Department of Oncology

University of Helsinki

Finland

RADIATION THERAPY OF HEAD AND NECK CANCER WITH SPECIAL EMPHASIS ON LOCOREGIONAL RECURRENCE AND ADVERSE EVENTS

Kauko Saarilahti

ACADEMIC DISSERTATION

To be publicly discussed, by permission of the Medical Faculty of the University of Helsinki, in the Auditorium of the Department of Oncology, Helsinki University Hospital, Haartmaninkatu 4, on November 5th, 2004, at 12 o'clock noon.

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SUPERVISED BY:

Professor Heikki Joensuu M.D.
Department of Oncology
University of Helsinki

and

Docent Mikael Kajanti M.D.
Department of Oncology
University of Helsinki

REVIEWED BY:

Professor Reidar Grénman M.D.
Department of Otorhinolaryngology - Head and Neck Surgery
University of Turku

and

Professor Pirkko Kellokumpu-Lehtinen M.D.
Department of Oncology
University of Tampere

OPPONENT:

Docent Antti Ojala M.D.
Department of Oncology
University of Tampere

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TABLE OF CONTENTS

1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Saarilahti K, Kajanti M, Kouri M, Aaltonen L-M, Franssila K, Joensuu H. Cyclin A and Ki-67 expression as predictors for locoregional recurrence and outcome in laryngeal cancer patients treated with surgery and postoperative radiotherapy. Int J Radiat Oncol Biol Phys 2003; 57: 986-995.

II Saarilahti K, Kajanti M, Lehtonen H, Hämäläinen T, Joensuu H. Repopulation during radical radiotherapy for T1 glottic cancer. Radiother Oncol 1998; 47: 155-159.

III Saarilahti K, Kajanti M, Atula T, Mäkitie A, Aaltonen L-M, Kouri M, Mäntylä M. Biweekly escalated, accelerated hyperfractionated radiotherapy with concomitant single-dose mitomycin C results in a high rate of local control in advanced laryngeal and hypopharyngeal cancer. Am J Clin Onc 2004; in press.

IV Saarilahti K, Kajanti M, Joensuu T, Kouri M, Joensuu H. Comparison of granulocyte-macrophage colony-stimulating factor and sucralfate mouthwashes in the prevention of radiation-induced mucositis. a double-blind prospective randomized phase III study. Int J Radiat Oncol Biol Phys 2002; 54: 479-485.

V Saarilahti K, Kouri M, Collan J, Hämäläinen T, Atula T, Joensuu H, Tenhunen M. Intensity modulated radiotherapy for head and neck cancer: evidence for preserved salivary gland function. Submitted.

2. ABBREVIATIONS

CDK	cyclin-dependent kinase
CDKI	cyclin-dependent kinase inhibitor
CHART	continuous hyperfractionated accelerated radiotherapy
CT	computed tomography
D_{50}	the dose for 50% complication probability
3-D	three-dimensional
DFS	disease-free survival
DMLC	dynamic multileaf collimator
DNA	deoxyribonucleic acid
EORTC	European Organization for Research and Treatment of Cancer
5-FU	5-fluorouracil
GM-CSF	granulocyte-macrophage colony-stimulating factor
Gy	Gray
ICRU	International Commission on Radiation Units
IMRT	intensity-modulated radiotherapy
MMC	mitomycin C
MRI	magnetic resonance image
OAR	organ at risk
PEG	percutaneous endoscopic gastrostomy
PET	positron emission tomography
PTV	planning target volume
RTOG	Radiation Therapy Oncology Group
SCCHN	squamous cell cancer of head and neck region

TNM	Tumour node metastasis
UICC	International Union Against Cancer
VAS	visual analogue scale
WHO	World Health Organization

3. INTRODUCTION

Based on the incidence and mortality data available, the global number of new cancers of the oral cavity, nasopharynx and other pharyngeal sites has been estimated to be 455 000, and the number of new laryngeal cancers 161 000 in the year 2000. Annually, these tumours are responsible for over 300 000 cancer deaths [1, 2]. Most patients (75 %) presenting with these tumours are men. In Finland in 2001 there were 306 new oral and pharyngeal cancers (excluding lip cancer) and 108 laryngeal cancers, and these cancers were the primary cause of death in 206 cases [3]. In the recent Eurocare-3 study, the survival of cancer patients patients diagnosed from 1990 to 1994 in 22 European countries was analysed [4, 5]. The European average 5-year relative survivals for head and neck cancers were strongly dependent on the primary site of the cancer, varying from over 60% for laryngeal cancer to only 23% for hypopharyngeal cancer. The 5-year survival figures for all head and neck cancers were 32.6% for men and 50.7% for women; in Finland, the corresponding figures were 42.9% and 57.5%. A survival advantage of ≥15% at 5 years was observed in women for four cancers arising in the head and neck areas, namely cancers of the salivary glands, tongue, oral cavity and oropharynx. These large differences were thought to be in part due to earlier diagnosis in women. Only for laryngeal cancer was a slight survival advantage noted for men.

Histologically, most head and neck cancers are squamous cell carcinomas. In laryngeal cancer, these comprise over 90% of all tumours; other histological types, including neoplasms with neuroendocrine differentiation, salivary gland adenocarcinomas and sarcomas, are rare. Approximately 90% of malignant tumours of the oral cavity and oropharynx are also squamous cell carcinomas. Most of the remaining 10% are carcinomas of minor salivary glands, which are futher subtyped according to the WHO classification [6]. Rarely, lymphomas, melanoma or sarcomas may

arise in the oral cavity. The nasopharyngeal carcinomas are divided into three main types: keratinizing squamous cell carcinoma, differentiated non-keratinizing carcinoma and undifferentiated carcinoma [7].

The strongest risk factor for squamous cell carcinoma of head and neck (SCCHN) region is cigarette smoking. Population-based studies of male cigarette smokers have reported relative risks of 3-13 for ever-smokers [8-10]. The risk associated with smoking is related to the number of cigarettes smoked per day and the length of exposure [10]. This has been suggested to be relatively higher in women than in men. The relative risk of light smokers, adjusted for alcohol consumption, has been estimated to be 1.6 in men and 3.0 in women, and the corresponding figures for heavy smokers 4.4 and 10.2 [9, 11]. In most case-control studies a relationship between smokeless tobacco products and oral cancer has been found [12], although not all investigators agree [13, 14]. Betel-quid chewing, practised in some Asian cultures, is associated with an increased risk of oral cancer[15]. Alcohol consumption is another risk factor for SCCHN, and there is evidence of a synergistic effect between smoking and alcohol consumption [10, 16]. The use of alcohol-based mouthwashes can also lead to an increased risk of oral cancer [17]. Human papillomavirus (HPV) has been identified as an risk factor of head and neck cancer (odds ratio 3.0-3.5) [18, 19]. Especially HPV types 16 and 18 are associated with an increased risk of SCCHN [20-22].

The main treatment modalities used in the treatment of head and neck cancer are surgery, radiotherapy and chemotherapy. Most early-stage head and neck squamous cell cancers can be cured by either radical surgery or radiotherapy. Also in advanced stages of head and neck cancer radiotherapy can be used as an alternative to surgical treatment. When radiation therapy is given for curative intent, a total dose of 65-70 Gy in 6-7 weeks is able to produce local control rates of 80-90% in T1 and T2 lesions [23-27]. However, the control rates are much lower in large (T3 or T4)

cancers [28-30], and for massive cancers doses ranging from 75 to 80 Gy or even more may be needed [31]. Escalation of tumour doses can produce higher local control figures, but at the price of increased radiation-induced toxicity [32, 33].

In the treatment of advanced stages of head and neck cancer, combined surgery and radiotherapy has been the most widely accepted standard therapy. Postoperative radiation therapy is usually considered when the risk of recurrence above the clavicles exceeds 10-20 %. The main clinical indications for postoperative radiotherapy are positive or close tumour resection margins, advanced primary tumour, presence of metastatic lymph nodes and possible extracapsular nodal or perineural spread [34, 35]. A total dose of 50 Gy with conventional fractionation (2 Gy per day and five fractions per week), is generally sufficient to control occult disease in 90% of cases [36]. In cases with marginal resection or extracapsular spread of nodal metastasis, doses in the range of 60-70 Gy are needed to prevent tumour recurrence. Another approach to combine surgery and radiotherapy is preoperative radiotherapy, where the primary intention is to prevent marginal recurrence, to control subclinical disease or convert inoperable tumours into operable ones. The main arguments presented against preoperative radiotherapy are the delay in surgery, loss of knowledge of the exact tumour extent at surgery, and possibly more frequent surgical complications following preoperative radiotherapy. In a prospective randomized study carried out by the Radiation Therapy Oncology Group (RTOG) in the 1970's that compared preoperative and postoperative radiotherapy in supraglottic laryngeal cancer and hypopharyngeal cancer, the local control rate was significantly better for the postoperative radiotherapy group, but overall survival was unaffected [37].

Only about one third of patients with squamous cell head and neck cancer present with T1 or T2 node-negative lesions. The remaining patients at diagnosis have locally or regionally advanced disease (T3-T4, N1-N3, M0). The survival rates for patients with advanced disease (stage III-IV),

have been disappointing with conventional therapy, within the range of 30-40%, and the majority of these patients will eventually die of cancer [38, 39]. There have been numerous attempts to make treatment of these tumours more effective by modifying fractionation in radiotherapy schedules and by combining radiotherapy with chemotherapeutic agents. In head and neck cancer patients, the ultimate cause of death is most often locoregional recurrence of cancer, and therefore, it is of utmost importance to develop treatment protocols that are able to produce maximal local control figures. Optimal fractionation in radiotherapy is still under investigation. In head and neck cancer, both hyperfractionationated and accelerated radiotherapy schedules have been able to produce better local control figures [40]. This has not, however, led to improved survival in these patients. Another approach has been to combine radical radiotherapy with chemotherapy, and evidence has emerged, that concurrent chemoradiotherapy can achieve not only better local control but also increased survival in advanced head and neck cancer [30, 41-45].

More intense combination therapy leads to intensification of acute radiation- and chemotherapy-related adverse events in normal tissues. In head and neck radiotherapy of special interest are acute radiation-related mucosal reactions because they render the patient more vulnerable to infections and malnutrition. Mucosal reactions are also a major cause of disruptions in the course of radiotherapy, which can lead to inferior local control [46, 47]. The efficacy of drugs used in the prophylaxis and treatment of radiation-related mucositis has been disappointingly low and more efficient medications are needed. In addition the delayed effects of radiotherapy, such as radiation-induced xerostomia, can be distressing for patients. Current radiotherapeutic techniques are able to reduce radiotherapy- related toxicity by lowering the dose to healthy normal tissues. In the prevention of radiation- induced xerostomia, some progress has also been achieved by radioprotectants such as amifostine [48]. The main limitations of the use of amifostine are adverse events, laborious use, and the costs involved.

During the past years technical developments in radiotherapy have been rapid. Computer-based three-dimensional radiotherapy planning programs have made the targeting and dose prescription more accurate. In the radiotherapy of head and neck cancer, modern patient fixation systems, such as thermoplastic masks and stereotactic head and neck immobilization devices, can minimize the effect of set-up errors in radiotherapy [49]. Novel radiotherapy techniques, such as conformal radiotherapy and intensity-modulated radiotherapy (IMRT), enable escalating the radiotherapy doses given to advanced tumours and simultaneously reducing the doses to healthy normal tissues, thus significantly improving the therapeutic ratio of radiotherapy [50].

Achieving better local control figures in head and neck cancer is not possible without intensifying treatment protocols, which in turn is associated with increased acute and late side-effects of radiotherapy. The aim of this work was to study the effect of fractionation and concomitant chemotherapy on the outcome of SCCHN and to enhance the therapeutic ratio of therapy by identifying ways to reduce radiotherapy-related toxicity in treatment of these cancers.

4. REVIEW OF THE LITERATURE

4.1. Locoregional recurrence of head and neck cancer following radiotherapy

The great majority of local failures in head and neck cancer occur within 2 years of treatment [51]. In a study by Eckardt *et al.*, 36.4% of all recurrences were detected within 1 year and 79.8% within 2 years of primary therapy [52]. In another study, local recurrences were noted in 26% (n=67) of 257 patients treated with surgery and radiotherapy for head and neck cancer, and in only 6 patients did the recurrences become evident after more than 2 years [53].

In a study by Pigot *et al.*, the exact site of failure was determined in 89 head and neck cancer patients with recurrent tumour after radiotherapy given with curative intent. Of the 73 patients who failed at the primary site, 71 (97%) did so within the site of the original tumour; only 2 patients developed marginal recurrence. Of the 30 patients with N1-3 nodal disease who later showed failure in the lymph nodes, 28 (93%) did so at their original site of disease [54]. Thus, when radiotherapy fails, it usually does so at the site of the primary tumour; this is in contrast to surgical failures, where marginal recurrences are common.

The frequency of locoregional recurrence in head and neck cancer is greatly affected by such tumour-related factors as size of the primary tumour and presence of nodal metastases. The probability of control of a tumour at a given radiotherapy dose level is a function of the number of clonogenic cancer cells that need to be eliminated [51]. In general, the clonogenic cell number is closely correlated with the tumour volume. Thus, with an increasing tumour volume, higher doses of radiotherapy are needed to ensure local control. For subclinical disease, a dose of 50 Gy given over 5 weeks is sufficient for achieving local control in 90-95% of cases. In tumours 2 to 4 cm in

diameter, a dose of 70 Gy in 7 weeks is recommended to achieve a 90 % probability of local control, and in massive tumours over 6 cm in diameter even larger doses (75 to 80 Gy) are needed [31].

The T stage of the primary tumour has proved to be an important determinant of local control. In a study by Johnson *et al.*, where T stages were grouped T1 to T3 vs. T4, the 36-month local control rates were 73% and 41%, respectively, ($p=0.03$). In the same study the N stage grouping of N0-1 vs. N2-3 was also associated with significant outcome difference (78% vs. 41%, $p= 0.009$). In a retrospective study of 476 patients with head and neck cancer, a multivariate analysis revealed that T stage, maximum tumour diameter, cancer differentiation grade, N stage, tumour site and overall radiotherapy treatment time correlated with locoregional control, in decreasing order of significance [55]. In a third large study consisting of 1000 patients with head and neck cancer, the incidence of local recurrence for T1 and T2 tumours was 28.9%, whereas in the T3 and T4 group it was 44.6% [52]. Muriel *et al.* reported the local control rate following surgery and postoperative radiotherapy to be 83% for T2 and 57% for T4 tumours, and within each stage, the N status was the major determinant for recurrence[56]. In several studies, the precence of nodal involvement has been described as the most important tumour-related prognostic factor for local control. The number of positive nodes [57, 58] and extracapsular spread of nodal metastasis [59, 60] have also been found to be important in predicting tumour recurrence. Nodal metastasis is not only an important determinant of local recurrence but also of distant metastasis. When Ellis *et al.* examined 455 head and neck cancer patients with nodal metastasis, a close correlation of the nodal stage and location was found with development of distant metastasis. Subsequent distant metastasis was observed in 11% of N1 patients, in 18% of N2 patients and in 27% of N3 patients, and the incidence was greater for those patients with metastatic adenopathy in the lower neck [61]. Some studies have reported a

close correlation between the total tumour volume measured in pretreatment CT scans and local control following radiotherapy for head and neck cancer [62, 63].

In postoperative radiotherapy for head and neck cancer, the radicality of surgery also has an important impact on the local control. Patients with positive surgical margins have greatly inferior local control figures [64-67], and larger postoperative radiotherapy doses are recommended for this group to achieve local control [53, 56]. Furthermore, perineural spread in histological specimens has an influence on local control in head and neck cancer treated by surgery and postoperative radiotherapy[68, 69].

The site of the primary tumour also has an important impact on local and distant tumour recurrence following therapy for head and neck cancer. This has been attributed to the rich vascular and lymphatic network present in certain areas, such as the base of the tongue, and absent in others such as the glottic larynx. Small, biologically aggressive tumours of the nasopharynx, the tonsilla fossa, base of the tongue or the pyriform sinus may present with extensive neck disease. In contrast, nodal disease is extremely rare in small T1 and T2 glottic cancers. The ultimate local control figures are generally low for primary tumour sites that tend to be associated with early nodal spread. The patterns of spread and the frequency of nodal metastasis at each T stage for different primary sites of head and neck cancers are presented in several textbooks of radiation oncology [70-72].

4.1.1. Therapy of recurred head and neck cancer

The main therapeutic modalities for local recurred head and neck cancer are surgery, irradiation and chemotherapy. After primary radiotherapy or combined modality therapy has failed, surgical

salvage is in most cases preferentially offered, if feasible. In small T1 and T2 laryngeal cancers, the local control figures after salvage surgery are high, 75% to 86% [25, 26, 73], and even for T3 laryngeal tumours the salvage rates are relatively high [74]. In more advanced, T4 tumours salvage surgery is less successful. Davidson *et al.* Reported a 3-year survival rate of 22% following treatment of recurrent advanced laryngeal cancer [75]. Although total laryngectomy is normally warranted in most patients with recurrent laryngeal cancer after failure of radiotherapy, in selected recurrent tumours (rT1 or rT2) larynx preserving surgery may be possible [73]. For most other primary sites, salvage surgery is far less successful, the salvage rates ranging from 24% to 32% for tumours of the oral cavity, including the tonsils, base of the tongue and the hypopharynx [76-78]. Salvage neck dissection may also be effective after radiotherapy in some patients [79-81]. Re-irradiation may occasionally be attempted after failure of primary radiotherapy. In studies on re-irradiation of recurrent local head and neck cancer, long-term survival has been reported in 13% to 20% of patients [82, 83]. In this situation, conformal radiotherapy, especially intensity modulated radiotherapy, may be valuable in restricting most of the re-irradiation dose to the site of relapse, thus allowing the radiotherapy dose to the recurred tumour to be escalated [84, 85]. Stereotactic radiotherapy can also be used in the treatment of small (rT1-rT2) recurrences and can, in selected patients, produce 1-year local control in up to 82% of the treated patients [86]. In a recent study by Ashamalla *et al*. radioactive gold implants were used to treat recurrent head and neck cancer, and a complete local control was achieved in 33% of recurrent tumours smaller than 2.5 cm in diameter. When the longest tumour diameter was greater than 2.5 cm, the local control rate was, however, only 11% [87]. Concomitant chemotherapy and radiotherapy has also been attempted, with results differing little from radiotherapy alone; some of the most recent data do, however, support the use of chemoradiotherapy [88]. In a study by De Crevoisier *et al.* a 5-year survival rate of 6% was achieved with reirradiation alone and a rate of 14% with concomitant treatment with 5-fluorouracil and hydroyurea [89]. With chemotherapy complete responses are achieved in less than 20% of

patients, and partial responses in 50-60%. Nevertheless, the duration of responses achieved with chemotherapy is usually only a few months, and the median survival time following chemotherapy is approximately one year [90-92].

As locoregional recurrence of cancer of the head and neck is associated in most instances with a relatively low probability of achieving a permanent cure, every attempt should be made to plan the primary treatment so that it is as effective as possible.

4.2. Factors influencing the frequency of locoregional recurrence following radiotherapy

Several tumour- and radiotherapy-related factors can cause local failure after radiotherapy of head and cancer. A crude geographic miss or tumour underdosage will unavoidably lead to local recurrence. With present radiotherapy technology, these are seldom causes of failure. Computer-based radiotherapy planning offers tools to estimate tumour doses with precision, and the use of modern immobilization devices and localization systems make gross geographic misses unlikely [49, 93-96]. Moreover, radiobiological factors related to cancer volume, hypoxia, tumour cell kinetics, intrinsic cellular radiosensitivity and tumour repair capacity may have an impact on therapy outcome [51, 97].

4.2.1. Pretreatment evaluation of head and neck cancer

Because the prognosis in head and neck squamous cell cancer is highly dependent on tumour stage, the treatment decisions are based on the exact staging of each tumour. In evaluation of prognosis in head and neck cancer, tumour node metastasis (TNM) staging is the most important. There is no

curative treatment for patients presenting with distant metastasis, and it is therefore important to rule this out at the time of diagnosis. Both primary tumour size and presence of nodal metastasis are associated with the ultimate outcome in head and neck cancer, the nodal stage being more important [98-101].

The initial staging of head and neck cancer usually includes physical examination, panendoscopy and computed tomography (CT) or magnetic resonance imaging (MRI) to evaluate the extent of the primary tumour and metastatic nodal disease [102]. Fine-needle aspiration cytology performed with ultrasound-guiding may provide additional information about the nature of enlarged lymph nodes, and it may be used to diagnose malignancy in small lymph nodes not found by other methods [103, 104]. In advanced stages of the disease, assessment of possible metastatic disease is also necessary [102]. Because of the importance of primary staging in head and neck cancer, new surgical and radiological methods have been developed for accurate staging. A sentinel node biopsy may be useful in staging of a clinically negative neck [105-108]. In one such study upstaging of the clinically negative neck occurred in as many as 5 (25%) out of the 20 patients with T1 cancer, 5 (42%) of the 12 patients with T2 cancer and in 5 (45%) of the 11 patients with T3 or T4 oral or oropharyngeal cancer [105]. Positron emission tomography (PET) as an initial staging procedure may also be helpful in this respect [109], and can also be used to identify multiple level disease in a clinically positive neck [110]. In a recent study by Schmid *et al.*, whole-body PET was able to assess lymph node involvement, distant metastasis and second primaries in a single study, and the authors concluded that even after a routine staging PET leads to a change in the treatment plan in 8% of patients [111].

In addition, the histological grade of differentation has been used to assess the clinical behaviour of a head and neck cancer. In a study of 1266 consecutive patients with head and neck cancer treated

with definitive or postoperative radiotherapy by Fortin *et al.*, grade was found to be a strong and independent factor associated with distant metastasis and survival [112]. In grades 1, 2 and 3, the respective distant metastasis-free survival rates were 97%, 92% and 76% for patients treated with radiotherapy, and 97%, 87% and 76% for patients treated with surgery and postoperative radiotherapy. However, the literature concerning the prognostic significance of the histological grade is contradictionary in head and neck cancer.

There is, however, significant prognostic variation within each TNM class and each tumour histological grade; some small cancers considered to have a low risk of recurrence eventually recur and, many large tumours can be cured with locoregional therapy only. Hence, prognostic tools more refined than tumour stage or grade are needed to estimate prognosis and help in treatment decisions.

4.2.2.. Fractionation in head and neck radiotherapy

Fractionation is one of the most important factors determining the outcome of radiotherapy. In conventional fractionated radiotherapy of head and neck cancer, a daily dose of 1.8-2.0 Gy is given 5 times a week for a total dose of 60 to 70 Gy over 6 to 7 weeks. The main types of unconventional fractionation are hypofractionation, hyperfractionation and accelerated fractionation. The radiotherapy can be given as a continuous treatment or with a mid-course pause (split-course radiotherapy). Hypofractionation in head and neck cancer has resulted in decreased tumour control and increased complication rates and has therefore been abandoned [113-115]. In split-course radiotherapy, the potential gain is the possible better oxygenation of the remaining tumour cells

after the treatment gap, leading to better radiosensitivity, and the split also gives time for the acute radiation-related side-effects to heal, thus making the treatment easier on the patient. Clinical experience, however, indicates that tumour control is consistently lower than with continuous radiotherapy [116-119]. Because of evidence indicating that hypofractionation and split-course radiotherapy are less beneficial in head and neck radiotherapy, the fractionation models left for further development are hyperfractionation and accelerated fractionation.

In hyperfractionation, multiple small fractions are given 2 to 3 times a day (e.g. 1.15 to 1.2 Gy twice a day), while the overall treatment time remains unchanged as compared with treatment times in conventional fractionation. The rationale is that because of the higher fractionation sensitivity of late-responding tissues the use of small fractions makes it possible to administer higher total doses within the tolerance of late-responding normal tissues. A higher biologically effective dose can be given to the tumour since the α/β ratio for the tumour is greater than that for the dose-limiting normal tissues. Hyperfractionation also gives a greater opportunity for cells that are in a radio-resistant phase to be redistributed to a sensitive phase during the radiotherapy, and the influence of tumour hypoxia may be reduced with small fractional doses [120]. Data from hyperfractionation regimens applying a 10 to 15% total dose increment over the standard 66 to 70 Gy have revealed a 10 to 15% improvement in local control rates without increasing the incidence of radiation-related late complications [32, 121].

In accelerated fractionation, conventional-sized fractions are given in shorter treatment span. The intention is to counteract clonogenic cell repopulation during the radiotherapy course. A briefer overall treatment time reduces chances of tumour cell repopulation, thus increasing the probability of local tumour control. Repopulation of the surviving clonogenic cells during fractionated radiotherapy is one of the most important factors determining the probability of cure. The radiation

dose needed to compensate for repopulation has been suggested to be larger than what is necessary to compensate for tumour growth if tumours maintained their preirradiation growth rate. This extra dose can be interpreted as accelerated repopulation of clonogenic tumour cells during radiation therapy[46, 122]. Accelerated repopulation has been estimated to begin about 2 to 4 weeks after the beginning of radiotherapy [123-125]. The dose needed to compensate for repopulation during fractionated radiotherapy in T2 and T3 cancers has been estimated to be 0.5-0.8 Gy/day [126-130]. Measurements of potential doubling times (T_{pot}) have shown a value ranging from 3 to 7 days for head and neck cancer [122, 131]. Because of the relatively short doubling times, several doublings of clonogenic cells could occur during a break of a few weeks in radiotherapy, and to compensate for this, the total dose should be raised significantly, especially as evidence indicates that the tumour repopulation rate might be even faster during treatment gaps than during the days of irradiation (required compensatory dose 0.75 vs. 0.2 Gy/day)[124].

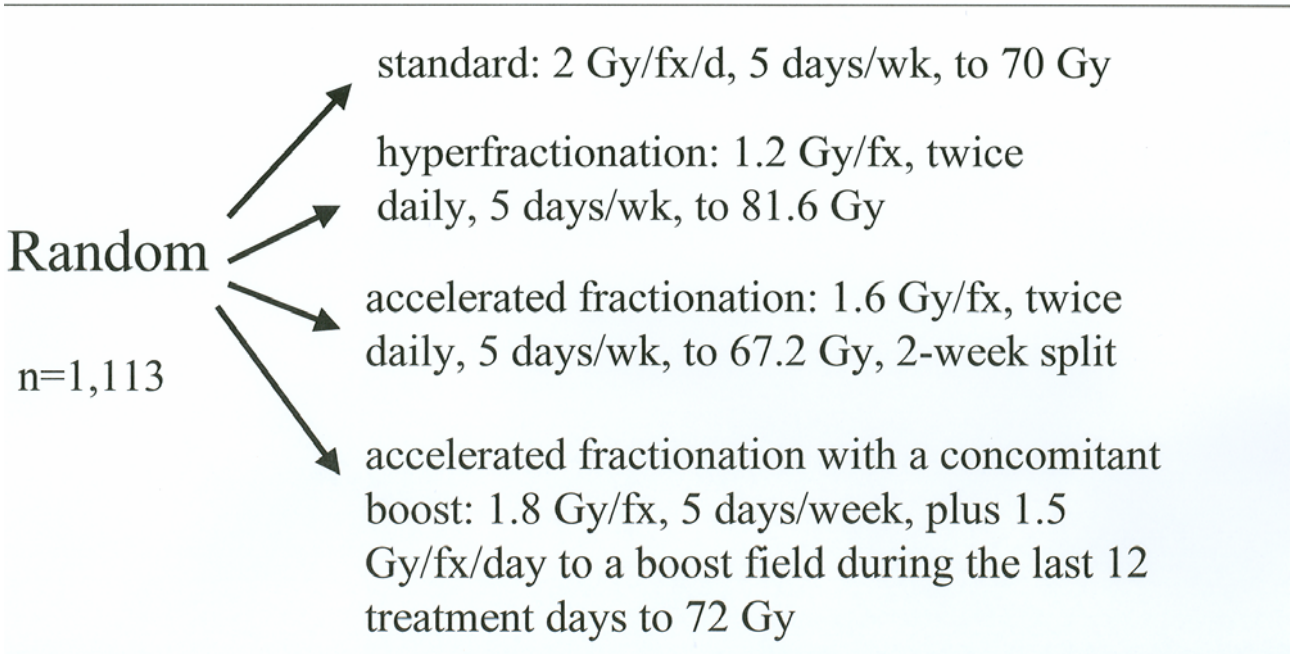
One of the most commonly used accelerated fractionation schedules is to give a concomitant boost to the primary lesion site along with a conventional fractionated radiotherapy programme [132-135]. Another method is to increase the amount of weekly fractions. In a Danish study, 1485 patients with head and neck cancer were randomly assigned to receive either 6 fractions per week in the experimental arm or 5 fractions per week in the conventional RT arm; a significantly better local control, but not overall survival, was observed in the accelerated treatment group [136].

Continuous hyperfractionated accelerated radiotherapy (CHART) is a hybrid form of accelerated radiotherapy. In a large trial of 918 patients with advanced head and neck cancer, the patients were randomized to receive either three 1.5-Gy daily fractions to a total dose of 54 Gy in 12 days or conventional radiotherapy in 2-Gy daily fractions to a total dose of 66 Gy in 6.5 weeks. No differences were found in local control, disease-free survival or overall survival [137]. Another

variant of accelerated radiotherapy is escalated, accelerated, hyperfractionated radiotherapy in which the daily fractions are escalated during the course of radiotherapy to counteract the accelerated repopulation of clonogenic cells [138, 139]. In this method, the radiotherapy is begun with, for example, 1.2 Gy twice a day, then after 2 weeks the fraction size is raised to 1.4 Gy, and after another 2 weeks to 1.6 Gy twice a day, such that the fraction size keeps rising towards the last weeks of radiotherapy, when repopulation of the remaining clonogenic tumour cells is thought to be fastest.

The largest clinical study so far that directly compared different modalities of fractionation in head and neck cancer is the RTOG 9003 study. The design of this clinical trial is presented in Figure 1.

Figure 1. RTOG phase III study to compare hyperfractionation and 2 variants of accelerated fractionation to standard fractionation in head and neck cancer (Fu et al. IJROBP 2000; 48: 7-16)



In this study, local control was significantly better in patients treated with hyperfractionation (54.4% vs. 46%, p=0.045) and those who received accelerated fractionation with a concomitant boost (54.5% vs 46%, p=0.050) than in patients treated with standard fractionation. No difference was observed between the accelerated split-course regimen and standard radiotherapy. There was a trend towards better disease-free survival in the hyperfractionated (p=0.067) and accelerated fractionation with concomitant boost (p=0.054) arms as compared with standard fractionation, but no significant difference was present in overall survival. Acute side-effects were significantly greater in all three groups of altered fractionation; in late effects, no difference was observed [40].

The main pitfall of the numerous trials with altered fractionation is that, although many of them have been able to produce better figures of local control, this has not led to improved survival in these patients. There are only a few clinical trials in which any effect on survival was found. One of these is the EORTC 22851 randomized trial, where a trend (p=0.06) towards better survival was observed in the accelerated treatment group [140]. Moreover, in most publications, altered fractionation has been found to be associated with increased acute radiation-induced reactions, such as mucositis [141-143], thus making the treatment more inconvenient for the patient and more complicated and demanding for the radiotherapy units.

Not only the overall radiotherapy treatment time, but also the time from surgery to the beginning of radiotherapy may influence the outcome of head and neck cancer patients. A systematic review by Huang *et al.* [144] summarized the results of seven studies involving a total of 851 patients treated by surgery and postoperative radiotherapy for head and neck cancer. The overview analysis showed that the locoregional recurrence rate was significantly higher among patients who received postoperative RT for head and neck cancer more than 6 weeks after surgery than among those treated within 6 weeks of surgery (OR = 2.89; 95% CI 1.60-5.21). The study found little evidence

suggesting that delay in initiation of RT might influence the risk of distant recurrence or the probability of long-term survival.

4.2.3. Assessment of tumour cell proliferation rate in radiation therapy of head and neck cancer

It would be interesting to determine whether the response to radiotherapy could be estimated based on tumour proliferative markers and whether the tumour repopulation rate during a RT course could be predicted by these markers. Theoretically, accelerated treatment is most important in tumours with a high proliferative capacity. One of the most studied factors in this respect is the Ki-67 proliferation antigen. The results from studies on the influence of Ki-67 antigen expression on radiotherapy response, local control and patient survival have been conflicting. Two studies have suggested that patients with a tumours containing a high proportion of Ki-67-positive cells (>20%) have better local control than those with a lower expression of Ki-67 [145, 146]. However, no such association after radiotherapy could be found in a study on patients treated for oral cavity cancer [147]. In assessing the prognosis of patients after chemoradiotherapy for head and neck cancer, Ki-67 was associated with overall survival but not with locoregional recurrence [148]. Lazaris *et al.* reported high Ki-67 expression to be associated with nodal metastasis and early recurrence in laryngeal cancer [149], in contrast to another study, where the Ki-67 index had no value in predicting treatment outcome in SCCHN [150]. Fortin *et al.* found tumour histological grade to correlate closely with Ki-67 expression levels [112]. Thus, the association of Ki-67 with local control and survival needs to be confirmed in a larger series of patients. Another measure of tumour proliferative capacity used is the tumour potential doubling time (T_{pot}). Research results have been variable, some showing none or borderline significance of T_{pot}, while others have indicated that the T_{pot} measurement is a strong prognostic parameter [151]. In a multicentre analysis reported by Begg

et al., head and neck cancers with a low labelling index (LI < 5%) had significantly better local control than tumours with a high LI [55].

Mutations or amplifications of genes that regulate cell growth or apoptosis may also lead to enhancement of the tumour growth capacity. Many SCCHNs express the receptor to epidermal growth factor (EGF) receptor (erbB1) in increased quantities. This may lead to aggressive growth and poor prognosis in patients with such tumours [152]. Mutations of the tumour supressor gene *TP53* have also been linked in some studies with poor prognosis in SCCHN; most studies have, however, found the *TP53* expression status to be of llimited prognostic significance [152, 153]. In a study by Hirvikoski *et al.* overexpression of p53 protein was associated with favourable disease-free and overall survival [154].

The sensitivity of cells to radiation varies widely depending on in which phase the cells are during radiation. Cells in the G2 and M phases are about three times more sensitive to irradiation than cells in the S phase [155]. The cell cycle is regulated by sequential activation of cyclin-dependent kinases (CDKs) by their partner cyclins. The cyclin-CDK complexes are involved in the initiation of both DNA replication and mitosis, and they control cell-cycle progression through various cell-cycle transition points [156-158]. In addition, CDK inhibitors (CDKIs) act to inhibit the cyclin-CDK complexes [159, 160]. In general, cell proliferation is balanced by stimulatory and inhibitory proteins and the transcription of genes regulating their synthesis. Evaluation of cyclins and their regulatory functions may aid in assessing prognosis and making treatment decisions in various human cancers.

Because of the close relation of the cell cycle phase of tumour cells to their radiosensitivity, attempts have been made to find correlations between regulators of the cell cycle and response to

radiotherapy, and eventually patient outcome. In head and neck squamous cell cancer, the most studied cyclin is the cyclin D1, and 35 to 64% of head and neck cancers have been reported to overexpress cyclin D1 or have *CCND1* (cyclin D1 gene) amplification [161-165]. Some studies have concluded that high expression of cyclin D1 is associated with poor outcome in laryngeal cancer [166-168]. Cyclins, cyclin-dependent kinases and the genes regulating their synthesis may also provide targets for cancer therapy in head and neck cancer. For example, the CDK inhibitor flavopiridol has inhibited transcription of cyclin D in preclinical studies, and induces a cell-cycle arrest at the transitions between the G2 and M phases and the G1 and S phases. Flavopiridol may induce p53-independent apoptosis [169] and is now being tested in clinical trials in head and neck carcinoma [170].

Cyclin A has a dual role in the control of the cell cycle. It is required for DNA replication during the S phase and is also expressed at high levels in the early mitotic phase [171, 172]. The cyclin A-CDK2 complex is a rate-limiting component required for cell entry into mitosis and the progression of the cell through mitosis until the late prophase, and the complex may be the target of the prophase checkpoint [173]. Cyclin A overexpression has been found to be an adverse prognostic factor in several cancers, including non-small-cell lung cancer [174, 175], breast cancer [176], colorectal cancer [177], renal cancer [178] and soft-tissue sarcomas [179]. In head and neck cancer, the role of cyclin A has not been defined, but cyclin A expression could potentially also be a useful prognostic marker in these tumours, facilitating treatment decisions. At present, however, none of the studied proliferation markers or cyclins has reached wide acceptance, and further studies are needed to define their roles in clinical practice.

4.2.4. Chemotherapy combined with radiotherapy in the treatment of head and neck cancer

Many chemotherapeutic agents have antitumour activity in the treatment of advanced SCCHN. As single-agent therapy, these drugs are generally able to generate response rates of 30% or less; the most extensively studied agents in this respect are cisplatin [180-182], carboplatin [183, 184], methotrexate [185, 186], 5-fluorouracil [181], ifosfamide [187] and the taxanes paclitaxel [188, 189] and docetaxel [189, 190]. The most commonly used combinations in the treatment of advanced head and neck cancer include cisplatinum and 5-FU [181, 182, 191], carboplatin and 5-FU [182, 183] and combinations of cisplatin or carboplatin with the taxanes [90, 189, 192]. Chemotherapy of advanced, locally or distantly recurrent, SCCHN may prolong survival by about only 10 weeks over the best supportive care alone [193]. Combination chemotherapy is able to produce higher response rates than single agents but does not improve survival as compared to single-agent therapy[193].

Chemotherapy can be combined with radiotherapy in several ways. In induction chemotherapy, the aim is to reduce the number of clonogenic cells and to cause reoxygenation of the surviving hypoxic cells, thus rendering tumours more easily controllable by radiotherapy [194]. The results from studies on induction chemotherapy have, however, generally been disappointing. The reasons for this may include accelerated repopulation of tumours induced by chemotherapy and selection or induction of drug-resistant cell lines cross-resistant to radiation [194]. Adjuvant chemotherapy designates a treatment modality where chemotherapy is given some time following radiotherapy. The main objective is to eradicate subclinical disseminated disease. In concurrent chemoradiotherapy chemotherapeutic agents are given simultaneuously with radiotherapy. In this form of therapy, the intention is to enhance both locoregional control and to eradicate possible micrometastatic disease outside the radiation fields.

Chemotherapeutic agents may enhance radiosensitivity of tumours by different mechanisms of action. Taxanes can block the transition of cells through mitosis, resulting in accumulation of cells

in the radiosensitive G2 and M phases of the cell cycle [195]. Nucleoside analogues, such as fludarabine and gemcitabine, become incorporated into radioresistant S-phase cells with subsequent elimination of these cells by apoptosis [196, 197]. The radiosensitizing properties of 5-fluorouracil (5-FU) are based on its incorporation into DNA and RNA, which leads to disruption of DNA and RNA function, and inhibition of thymidylate synthetase function and of direct incorporation of 5-FU into DNA. The effect of platinum-based compounds is based on inhibition of DNA synthesis, inhibition of transcription elongation by DNA interstrand cross-links and inhibition of repair of radiation-induced DNA-damage [194]. Some chemotherapeutic agents, such as mitomycin C and tirapazamine, are known to sensitize hypoxic cells to radiation [198, 199]. Topoisomerase I inhibitors (e.g. irinotecan and topotecan) inhibit the repair of radiation-induced DNA strand breaks and also redistribute cells into the more radiosensitive G2 phase [200, 201].

Despite numerous randomized trials, the impact of adjuvant or neoadjuvant chemotherapy as an adjunct to locoregional treatment of head and neck cancer has been disappointing. In a recent meta-analysis by Pignon *et al.*, which consisted of 63 randomized trials conducted between 1965 and 1993 and included 10 741 patients with locoregional squamous cell cancer of the oropharynx, oral cavity, larynx or hypopharynx, no significant survival benefit was found with adjuvant or neoadjuvant chemotherapy [41]. In contrast a significant ($p<0.0001$) survival benefit of 8% at 5 years was observed with concomitantly given chemotherapy. The value of the concomitant chemotherapy was assessed from 14 very heterogeneous trials which included only 11% of the patients in the meta-analysis; thus, the size of the benefit remained uncertain. When the concomitant chemotherapy trials were grouped according to the number of chemotherapy agents used, the effect of concomitant chemotherapy turned out to be significantly greater with multiagent chemotherapy than with single-agent chemotherapy (hazard ratio 0.69 vs. 0.87, $p<0.01$).

Since 1993, several randomized trials on concomitant chemoradiotherapy of SCCHN have been published (Table 1), and the results suggest markedly improved anti-cancer efficacy [30, 42-45, 202]. The results of these studies are summarised in Figure 2 with respect of the local control and in Figure 3 with respect of overall survival.

Table 1. Recent (1993-) studies comparing RT to concurrent chemo-RT in squamous cell head and neck cancer

Study	N	Criteria	Therapy
Wendt 1998	298	stage III/IV, unresectable	RT vs. RT/cis/FU
Adelstein 2003	295	stage III/IV	RT vs. RT/cis vs. split RT/Cis/FU
Calais 1997	226	oropharynx stage III/IV	RT vs. RT/carbo/FU
Jeremic 1997	159	stage III/IV	RT vs. RT/cis vs. RT/carbo
Jeremic 1997	136	stage III/IV	RT vs. RT/cis
Brizel 1998	122	T3, T4, N3, base of tongue T2N0	RT vs. RT/cis/FU +PFx2
Bachaud 1997	83	stage III/IV, extracapsular spread at surgery	RT vs. RT/cis

Figure 2. 2- or 3-yr local control rates in 7 recent randomized trials comparing RT to RT+concomitant platin ± 5-FU in stage III or IV H&N cancer

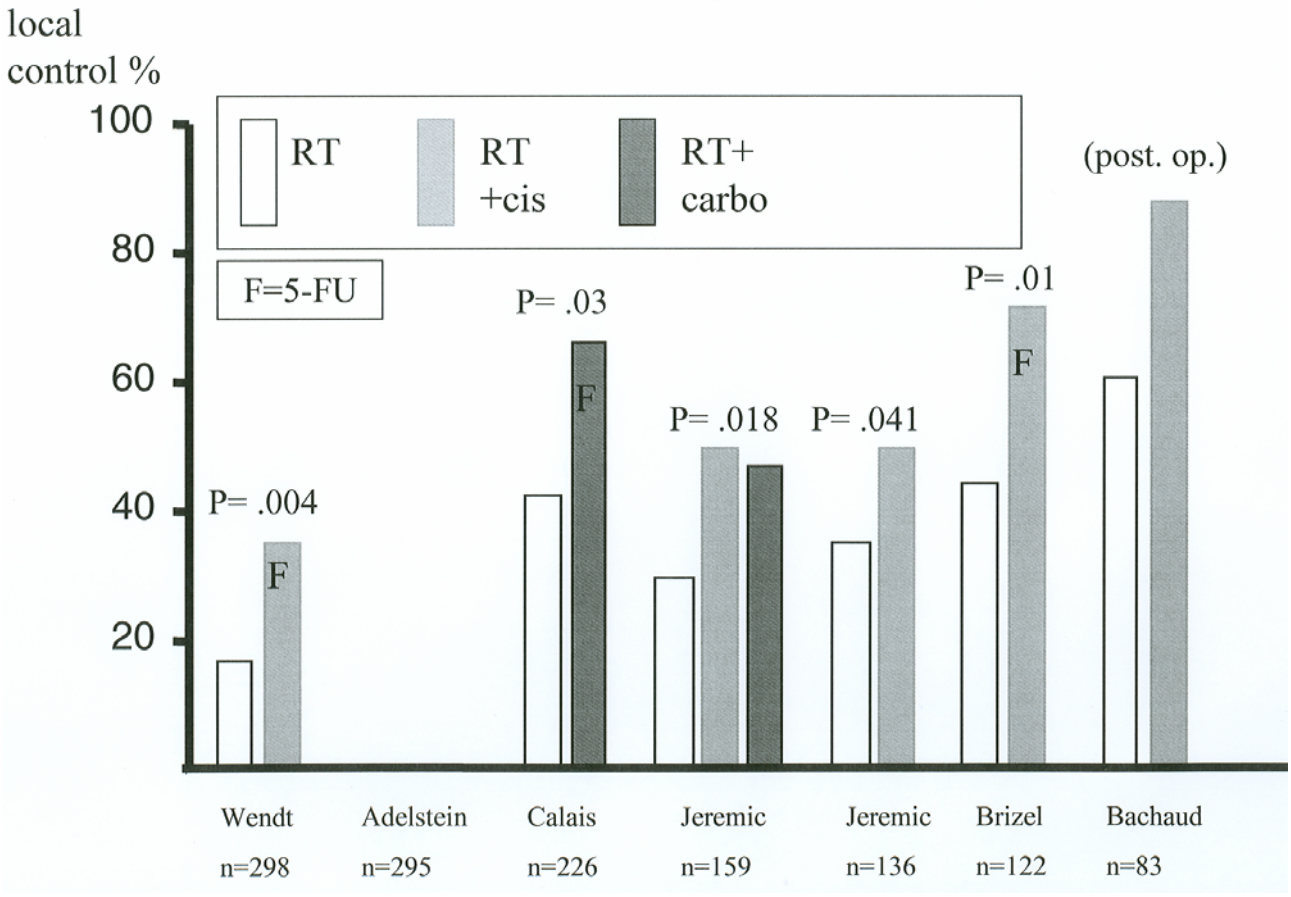
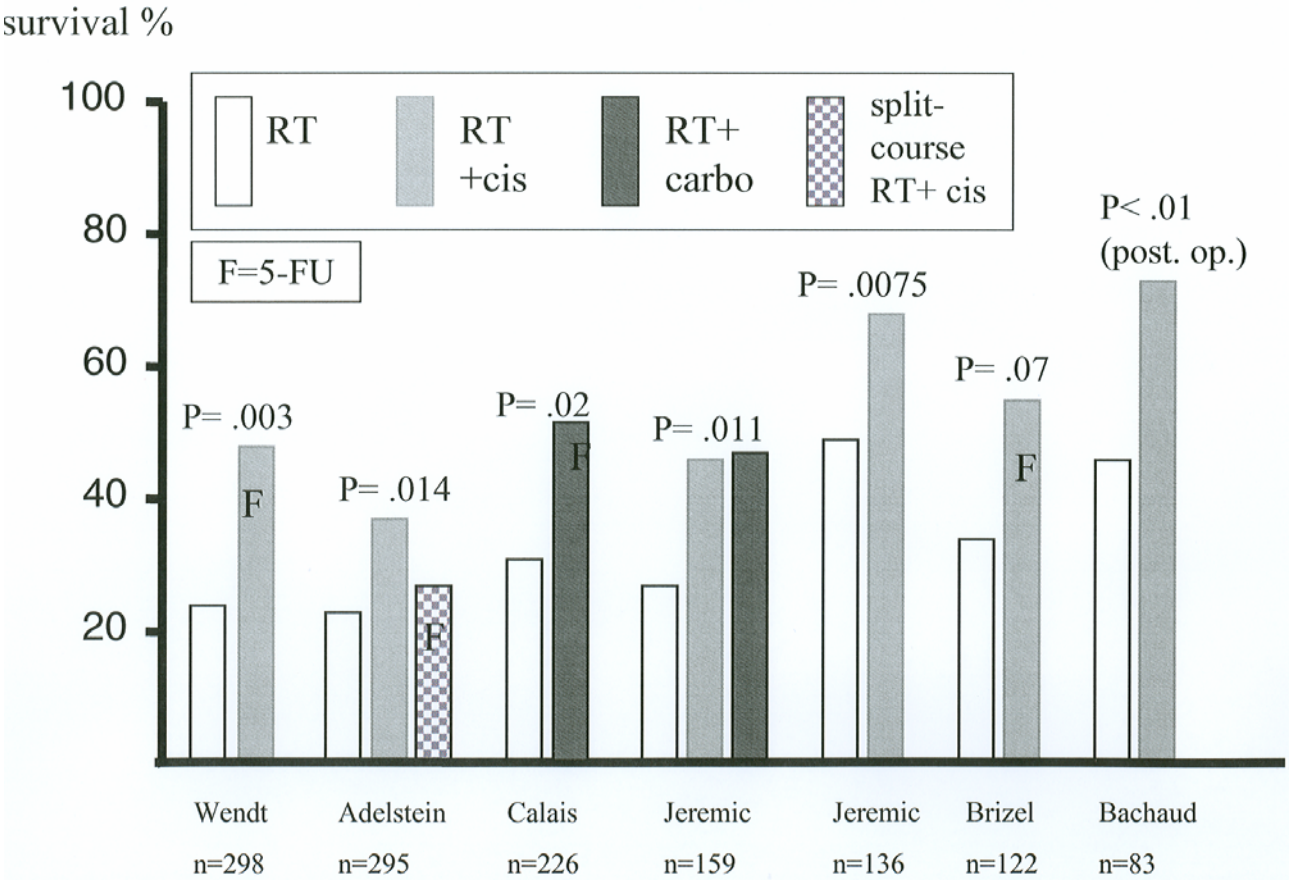


Figure 3. 2- or 3-yr overall survival in 7 recent randomized trials comparing RT to RT+concurrent platin ± 5-FU in stage III or IV H&N cancer



In a prospective randomized multicentre trial by Wendt *et al.*, 298 previously untreated patients with locoregionally advanced head and neck cancer were treated with either radiotherapy alone or simultaneous radiotherapy plus chemotherapy consisting of cisplatin, fluorouracil and leucovorin administered three times during the course of radiotherapy. Radiotherapy was identical in both arms (70.2 Gy given in 1.8 Gy fractions). Concomitant chemotherapy resulted in improved local control (48% vs 24%) and survival (36% vs. 17%) [42]. In another trial reported by Calais *et al.*, a total of 226 patients with advanced oropharyngeal carcinoma participated in a phase III multicentre, randomized trial comparing radiotherapy alone with radiotherapy plus concomitant chemotherapy. Radiotherapy was identical in the two arms, consisting of conventional fractionation up to 70 Gy in 35 fractions. In the experimental arm, patients received during the course of radiotherapy three 4-day cycles of carboplatin and 5-fluorouracil. In this trial, too, a significant improvement in both

local tumour control and overall survival was observed [202]. In a third large trial, reported by Adelstein *et al.,* 295 patients with unresectable SCCHN were randomly assigned to receive: 1) single daily fractionated radiation (70 Gy, 2 Gy/day), 2) identical radiation therapy with concurrent bolus of cisplatin, given on days 1, 22 and 43 of the radiotherapy course or 3) a split course radiation, where three cycles of concurrent fluorouracil and bolus cisplatin chemotherapy were given; two of the cycles were given concomitantly with radiation. In this trial, the addition of concurrent high-dose, single-agent cisplatin to conventional once daily fractionated radiation significantly improved survival, although it also increased acute toxicity. The loss of efficacy resulting from split-course radiation was not offset by multiagent chemotherapy or by midcourse surgery proved in the split-course arm [30].

A trial published by Brizel *et al.* in 1998, compared the efficacy of hyperfractionated irradiation plus concurrent chemotherapy (combined treatment) to hyperfractionated irradiation alone. A total of 122 patients with advanced head and neck cancer were randomized to receive either hyperfractionated irradiation (a total dose of 75 Gy; 1.25 Gy given twice a day) or hyperfractionated therapy (70 Gy; 1.25 Gy given twice a day) and 5 days of treatment with cisplatin and fluorouracil during weeks 1 and 6 of irradiation. Two cycles of cisplatin and fluorouracil were given to most patients after the completion of radiotherapy. At 3 years, both overall survival (55% vs 34%, p=0.07) and locoregional control of the disease (70% vs 44%, p=0.01) were superior in the combined therapy group [44].

Postoperatively given concurrent chemoradiotherapy improves local control and survival among high risk patients with resected head and neck cancer. In a study reported by Cooper *et al.,* 459 patients with head and cancer were randomly assigned to to receive either 1) postoperative radiotherapy alone or 2) postoperative radiotherapy and cisplatin 100mg/m^2 on days 1, 22 and 43

during the radiotherapy course in the experimental arm. High risk characteristics for recurrence were defined as presence of one or more of the following features: histologic evidence of cancer invasion to two or more regional lymph nodes, extracapsular extension of the nodal disease, or microscopically involved mucosal margins of resection. Both locoregional control and disease free survival were significantly better in the combined therapy group [203]. Similar results were obtained from another randomized trial by Bernier *et al* [204].

Although not leading to improved survival rates, induction chemotherapy followed by radiotherapy may have some role in attempts at organ preservation. In a study by the Veterans' Affairs Laryngeal Cancer Study Group, a total of 332 patients were randomly assigned either to the standard therapy arm consisting of laryngectomy with postoperative radiotherapy or to an experimental arm consisting of three cycles of cisplatin and fluorouracil, followed by RT. The larynx was preserved in 64% of patients in the experimental group. No difference was found in survival [205]. In another study concluded by the EORTC Head and Neck Cancer Co-operative Group, the larynx was preserved in 42% of patients receiving induction chemotherapy followed by radiotherapy for hypopharnyx cancer [206]. Neither of the studies mentioned above had a radiotherapy-only arm, which makes it difficult to assess, whether the same results could have been achieved without chemotherapy. In a recently published randomized study by Forastiere *et al.*, radiotherapy given with concurrent chemotherapy was compared to radiotherapy alone or to induction chemotherapy followed by radiotherapy in patients with laryngeal cancer. After a follow-up of two years, the rate of locoregional control and the proportion of patients with preserved larynx was higher in the chemoradiotherapy group than in the other two groups [207]. On the basis of this study one can question, whether induction chemotherapy followed by radiotherapy can be recommended even in laryngeal cancer. Since the chemoradiotherapy protocols have improved locoregional control rates, distant metastasis failures are becoming a more important cause of treatment failure than local

recidives. As a consequence, renewed interest has arisen on the possibility of eradicating micrometastasis by modern induction chemotherapy or by adjuvant chemotherapy in head and neck cancer patients, and thus, in improving overall survival [193]. The concept of induction chemotherapy, however, remains experimental.

Results from both *in vitro* and *in vivo* studies have suggested that mitomycin C is preferentially cytotoxic for hypoxic cells as compared with well-oxygenated cells [198, 208, 209]. Theoretically, this might be of value when treating advanced head and neck tumours, which often contain poorly oxygenated, radioresistant clonogenic cells.

Data from randomized studies suggest that infusions of mitomycin C (MMC) during radiation therapy may improve the outcome of fractionated radiotherapy in head and neck cancer (Table 2).

Table 2. Recent trials comparing RT vs. RT + concurrent mitomycin C (MMC) in head and neck cancer

Study	N	Therapy	Survival	Local control
Grau 2003	558	RT vs. RT/MMC	29% vs. 31%	18% vs. 21%
Dobrowsky 2000	239	RT vs. accelerated RT vs. accelerated RT/MMC	29% vs. 31% vs. 51%*	31% vs. 32% vs. 56%*
Zakotnik 1998	64	RT vs. RT/MMC/blm	7% vs. 26%	N.A.
Budach 2000	384	RT vs. RT/MMC/FU	45% vs. 54%*	45% vs. 61%*

Two consecutive randomized trials were performed at the Yale University School of Medicine between 1980 and 1992. A total of 203 patients were enrolled in these trials. Intravenous MMC (15 mg/m²) or MMC (15 mg/m²) and dicumarol were given as an adjunct to conventional fractionated radiotherapy (the total cumulative dose ranged from 60 to 68 Gy). A significant benefit was achieved in the MMC arms with respect to cause-specific survival (74% vs. 51%; p=0.005), local recurrence-free survival (85% vs. 66%; p=0.002), and loco-regional recurrence-free survival (76% vs. 54%; p=0.003). No significant difference in overall survival was found between the MMC arms and radiation alone [210]. Another large randomized study by Dobrowsky and Naudé supports the efficacy of MMC given concomitantly with fractionated radiotherapy. In this study, 239 patients with squamous cell cancer originating in the head and neck region were randomized to receive either 1) conventionally fractionated radiation therapy to 70 Gy in 35 fractions given over 7 weeks, 2) continuous hyperfractionated accelerated radiotherapy to a total dose of 55.3 Gy in 33 fractions over 17 consecutive days (V-CHART) or 3) V-CHART with concomitant administration of 20 mg/m² MMC on day 5 of treatment. A significant improvement in local tumour control and survival was found in the accelarated fractionated treatment plus MMC arm as compared to the two RT arms, but no difference was observed between the two RT arms [211]. In another large trial, 212 patients with previously untreated advanced squamous carcinoma of the larynx or hypopharynx were randomized to receive either 1) initial treatment with radiotherapy, 50 Gy in 20 fractions given over 28 days, or 2) to split-course radiotherapy, where 50 Gy was given in 20 fractions with a 4-week break in the middle of the radiotherapy course and with concurrent MMC given on days 1 and 43, and 5-FU continuous infusions given on days 1 to 4 and days 43 to 46 of the radiotherapy course. The result of the trial showed no advantage in local control or survival for the experimental treatment arm of split-course radiotherapy and concurrent chemotherapy with MMC and 5-FU

compared with radiotherapy alone [212]. This is in line with the observation from the previously mentioned trial by Adelstein *et al.* [30], where no effect was achieved with chemotherapy in the split-course radiotherapy group. In a recent multicenter trial reported by Grau *et al.* that compared fractionated radiotherapy (66 Gy in 33 fractions) with or without MMC 15 mg/m² given at the end of the first week of treatment, no benefit was observed with concomitant mitomycin except in N0 patients, where locoregional control was significantly enhanced [213].

In concurrent chemoradiotherapy of head and neck cancer, the best documented single agent is at present cis-platinum, and the most studied drug combination is cis-platinum combined with 5-FU. In future chemoradiotherapy trials, potential new drugs should probably be compared to these agents.

4.2.5. Advances in the radiation therapy delivery techniques

Developments in imaging technologies, including computed tomography (CT) and magnetic resonance imaging (MRI), together with rapid advancements in computer systems have greatly improved radiotherapy planning procedures recently. CT and MRI are capable of providing a full 3D model of the anatomy, thus enabling tumour volumes and their relationships to normal, healthy tissues to be estimated more accurately. Novel functional imaging techniques, such as positron emission tomography (PET), may in selected cases also be helpful in delineating radiotherapy target volumes [214-218]. By using fusion of PET and CT/MRI images, it may be possible to enhance treatment accuracy even further [219].

Three-dimensional CT-based treatment planning enables the use of conformal radiotherapy (CRT), in which the dose distribution to the target volume is adjusted to conform to the shape of the tumour to better avoid irradiation of the critical normal tissues. With this method, the dose to the tumour can be increased, and doses received by normal tissues reduced. To improve the conformality of the dose distribution, conventional beam modifiers, such as wedges or compensating filters, can be used. The new generation of linear accelerators are equipped with computer-controlled multileaf collimators (MLCs), which enable irregular shaping of treatment volumes and are thus very useful in 3D-CRT [220].

Intensity-modulated radiotherapy (IMRT) is a novel form of CRT planning and delivery technology. It represents one of the most important technical advances in the development of radiotherapy. IMRT is based on the use of optimized non-uniform radiation beam intensities incident on the patient. IMRT allows production of concave and irregular target volume dose distributions, and thus has the potential to reduce the volume of healthy tissues irradiated to a high dose [50]. The basis for development of IMRT was the rapid advancement in computer hardware and software techniques during the past decade. Modern IMRT planning is generally based on inverse treatment planning to achieve an optimal dose distribution in the target volume, while simultaneously sparing sensitive normal structures [50, 221, 222]. In radiotherapy planning, the target volumes and the organs-at-risk must first be defined similarly as in conventional 3D-CRT planning. The dose constraints for all prescribed volumes must then be defined [221, 223, 224]. When this has been accomplished, a computer optimization system is used to adjust the beam parameters to achieve the desired outcome [50]. In IMRT of head and neck tumours and cervical node areas 5 to 9 coplanar fields are usually used [225-232], although occasionally as few as three fields may be sufficient [229]. The radiation distribution within the target volume is most often

accomplished by multileaf collimators by programming the leafs to move dynamically during the radiation, thus modifying the radiotherapy dose at different points of the target volume [233-240].

Clinical implementation of the IMRT technique requires novel methods for quality control of the equipment and for verification of the treatment plans. For quality assurance, the dynamic multileaf collimator (DMLC) boundaries are verified on a localization port film, the leaf motions are verified to produce the planned dose distribution, the dose distributions produced by DMLC are verified to produce the dose distribution that is consistent with the treatment plan, the leaf motions are compared with those implemented for the treatment and the initial and final positions of the MLC for each field are confirmed, and the actual doses are verified by *in vivo* dose measurements [241-243]. In a study by Van Esch *et al.*, the final correspondence between the calculated and measured dose was found to be satisfactory in all five participating radiotherapy centres [243]. During the actual treatment the most important cause for error was found to be in patient positioning rather than dosimetry. In IMRT of head and neck cancer, keeping the patient's position unchanged throughout the entire treatment course is thus critical. This can be accomplished by using a conventional thermoplastic head and neck fixation mask or a stereotactic head and neck immobilization device. Patient positioning and field localization must be confirmed by repeated simulations during the treatment course.

Clinical benefits of IMRT are expected to be most pronounced at body sites where sensitive normal tissues surround or are located close to a target with a complex 3D shape. In the radiotherapy of head and neck cancer, the irradiation doses needed for tumour control are often much higher than the tolerance of the surrounding structures such as the spinal cord, the optic nerve, the eyes and the salivary glands. IMRT provides a tool to reduce the dose to such sensitive normal structures or, alternatively, to target dose escalation at a given level of normal tissue damage [228, 244-246].

IMRT has been successfully used in the treatment of head and neck cancer. In nasopharyngeal cancer, excellent tumour target coverage with higher tumour doses has been achieved than using traditional 3D planning with significant sparing of the salivary glands, the spinal cord, the brain stem and other critical normal tissues [244, 245, 247-249]. In most of these studies, the follow-up is not yet sufficiently long to allow comparisons with the results achieved by normal CRT, but good local and locoregional control has achieved with IMRT [249, 250]. In the treatment of parotid neoplasms, IMRT can be used to lower the irradiation doses to the brain, the spinal cord, the cochlea and the oral cavity. The dose to the contralateral parotid gland delivered with IMRT is dependent on the field arrangements and can be minimized by optimization of beam directions [225, 229]. IMRT is also useful in minimizing the radiation dose to the optic pathways in the treatment of maxillary sinus tumours and other paranasal sinus tumours, as well as orbital and paraorbital tumours [226, 251-253].

4.3. Radiotherapy-related adverse events following treatment of head and neck cancer

4.3.1. Acute adverse effects

The adverse effects of radiotherapy can be chronologically divided into acute and late effects. Acute adverse effects are defined as changes in tissues or associated symptoms noted within 90 days from the date of initiation of radiotherapy [254, 255]. The most widely used grading system for acute radiation-induced adverse events until recently has been the RTOG Acute Radiation Morbidity Scoring Criteria [255]. In this classification, acute morbidity is classified from grade 0 (no change

from the baseline) to severe, grade 4 reactions. Since 1997, the Common Toxicity Criteria Version 2.0 has provided a more comprehensive classification of toxicities of all treatment modalities, including radiotherapy [256].

The acute reactions most often encountered in radiation therapy of head and and neck cancer are acute mucositis and radiation-induced skin reactions. Acute mucositis is discussed in more detail in section 4.3.3. Acute changes in the skin begin with erythema usually at the cumulative dose of 20 to 40 Gy, then progress from dry desquamation to moist desquamation as the total cumulative dose increases to 45 to 50 Gy. Moist desquamation either heals within 50 days following radiotherapy or progresses to necrosis if the dose is unacceptably large [257]. The doses required to produce a 3%, 5%, or 50% incidence of skin necrosis at 5 years within a $30cm^2$ field size have been estimated to be 51 Gy, 55 Gy, and 70 Gy, respectively [257]. Irradiation of the taste buds can lead to disturbances in the ability to taste. An estimation has been made from animal models that approximately 20 to 30% of the taste cells are destroyed within each taste bud after fractionated irradiation to a total dose of 20 Gy. The cells of the taste buds are capable of repopulating at least within the first 4 months after treatment in most cases, but some permanent impairment may remain [258]. In a study by Emami *et al.*, an estimated TD_5 (a dose at which 5% of the patients have the complication) value for oedema of the laryngeal mucosa was 45 Gy, and the TD_{50} value for the same end-point was 80 Gy when the entire larynx was irradiated [259].

Increased acute toxicity has been linked with protocols that use accelerated radiotherapy or concurrent chemotherapy; in most studies, grade III and IV mucosal toxicity has been the most problematic [260]. Increased treatment-related toxicity calls for research on toxicity relieving therapies [261].

4.3.2. Late adverse events

Late effects are defined as changes in tissue or associated symptoms that occur more than 3 months from the beginning of radiotherapy [255]. In grading of these adverse effects, the RTOG/EORTC late radiation morbidity scoring scheme is commonly used [255]. The risk of radiation-induced late complications of various organs is highly dependent on the dose given and the treatment volume and fractionation used. For conventional fractionation, the minimal tolerance dose of each tissue is defined as $TD_{5/5}$, which represents the dose of radiation that would cause no more than a 5% severe complication rate within 5 years after radiotherapy [254]. Correspondingly, the $TD_{50/5}$ value is the dose of radiation that would cause a 50% severe complication rate at 5 years. Care should be taken when using unconventional fractionation schedules, because the $TD_{5/5}$ values for late normal tissue damage are valid only for conventional fractionation. To express an equal biological effect produced by different fractionation schemes, isoeffect lines have been generated [254]. Slopes for tumour curability and for normal tissue late effects have been calculated. In general, the slope for tumour curability is less steep than that for normal tissue reactions [254].

When estimating the late radiation effects, an organ can be considered to consist of multiple functional subunits (FSUs) that are arranged serially or in parallel [254]. In serially arranged organs, such as the spinal cord, damage to one portion of the organ may render the entire organ dysfunctional. In organs with parallel function, such as salivary glands, the surviving FSUs operate independently of the damaged group, and thus, organ function is maintained if the proportion of the functioning FSUs is large enough [262].

The capacity for repair of sublethal injury is the most important biological phenomenon influencing the fractionation response of tissue [97]. Slowly responding tissues consistently show a greater capacity for repair than rapidly responding tissues [263]. Large dose fractions have been demonstrated to be more harmful for late-responding tissues, and thus, a therapeutic gain may be achieved by hyperfractionation [97]. When using hyperfractionation or accelerated fractionation, the interfraction time period must be at least 6 hours to allow complete repair of sublethal damage in late-responding tissues. In a phase I-II RTOG trial on hyperfractionation a short interfraction interval (<4.5 hours) was found to be a major determinant of late effects when twice-a-day irradiation schedules were used [264].

Most late effects develop within the first 3 years following radiotherapy for head and neck cancer, and a few progress beyond 3 years [260]. Data derived from RTOG trials indicate that 85% of patients who received conventional radiation alone experienced some form of late toxicity. Approximately 12% suffered from grade 3 or 4 reactions, the most common of which were xerostomia, dysphagia and laryngeal toxicity [260]. Some mucosal atrophy and loss of mucosal mobility are common after conventional fractionated radiotherapy to a total dose of 60 to 70 Gy, but bone exposure seldom occurs unless dose delivery is accelerated or the total dose exceeds 70 Gy in 7 weeks [265]. The $TD_{5/5}$ for telangiectasia of the skin is about 45 Gy. Higher doses lead to an increased incidence of telangiectasia, fibrosis and atrophy [257]. The $TD_{5/5}$ and $TD_{50/5}$ values for laryngeal cartilage necrosis are estimated to be 70 and 80 Gy, respectively. Another possible severe late complication of head and neck radiotherapy is mandibular osteoradionecrosis; the $TD_{5/5}$ value for this complication, when the entire organ is irradiated is 60 Gy [259].

The late effects of radiation to the central nervous system must also be taken into account. The 5% incidence of radiation myelopathy has been suggested to be between a total dose of 57 and 61 Gy

with conventional fractionation [266]. The severe complication rate of the spinal cord using the conventional dose limit of 40 to 45 Gy given in 1.8-2 Gy fractions over 4 to 5 weeks is practically nil. Brain necrosis is seldom noted at doses of 60 Gy or less with conventional fractionation in adults. Neurocognitive changes may, however, take place at lower doses, especially in children [266]. When the hypothalamic-pituitary axis is included in the treatment field, neuroendocrine disturbances are observed at doses exceeding 40 Gy [267]. Late ocular, vestibular and hearing adverse effects need also be considered when planning radiotherapy for head and neck cancer [268, 269].

4.3.3. Radiation mucositis

Oropharyngeal mucositis is the most common and clinically significant acute adverse effect of radiotherapy for head and neck cancer. With conventional fractionation, the first signs of mucositis normally appear during the second week of radiotherapy and progress towards the end of radiotherapy from enanthema to spotted or confluent pseudomembranous mucositis [270-272]. Recovery occurs within 2.5 to 3 weeks after completion of radiotherapy, and within one month the mucosa heals in 90 to 95% of patients [260, 271]. Acute mucosal reactions cause pain, with concomitant difficulties in swallowing and speaking. Difficulties in eating may lead to worsening of the nutritional status and weight loss, and mucositis also predisposes to local and systemic infections. Severe mucositis is the most common cause for interruption of the radiotherapy course for head and neck cancer, which in turn can lead to significant loss of local tumour control probability [47, 270].

With hyperfractionated and accelerated radiotherapy, mucositis appears earlier and tends to be more severe than with conventional fractionation [137, 271-273]. The incidence of grade ≥3 mucositis increased from 20 to 50% to 66 to 86% in several trials where accelerated fractionation was used [260]. In chemoradiotherapy trials, the most problematic acute toxicity is also increased mucosal toxicity [260]. Drugs that often cause mucosal side-effects include 5-FU, capecitabine, methotrexate, bleomycin and doxorubicin. In contrast, drugs such as cisplatin and carboplatin seldom cause mucosal problems as single agents and are therefore preferable as chemoradiotherapy agents because they have little overlapping toxicity with ionizing radiation [265]. Efforts have been made to counteract the increased toxicity associated with intensified treatment protocols with various toxicity ameliorating drugs [261].

4.3.4. Xerostomia

One of the most common and distressing long-term adverse effects of head and neck radiotherapy is permanent xerostomia resulting from salivary gland damage. Xerostomia predisposes to infections and dental caries and disturbs swallowing and speech [265, 274]. Saliva is normally produced at a rate of 1000 to 1500 mL per day. Over 90% of this amount is secreted by three pairs of major salivary glands; the parotid, the submandibular and the sublingual glands. The minor salivary glands scattered over the mucosal surfaces of the mouth and pharynx account for less than 10% of the total salivary production [275]. In one study the submandibular glands produced 69% of unstimulated salivary flow and the parotid and sublingual glands 26% and 5%, respectively, whereas the parotid glands produced two-thirds of stimulated saliva [275]. Less than 0.1 mL/minute of unstimulated salivary flow and 0.5 to 0.7 mL/minute of stimulated salivary flow are generally considered as abnormally low [276].

The most important treatment-related factors that contribute to radiation-induced xerostomia are the volume of the salivary glands included in the irradiation fields and the total dose [265]. Parotid gland salivary flow is markedly reduced when a cumulative dose of 30 to 50 Gy is given using conventional fractionation [265, 277-282]; this dose level is often exceeded in conventional CRT of head and neck cancer.

4.4. Prevention of radiotherapy-associated mucositis and xerostomia

4.4.1. Prevention of mucositis

The progress made in studies of altered fractionation and chemoradiotherapy in head and neck carcinoma offers tools for achieving better local control and, with chemoradiotherapy, also better overall survival than that achieved with conventional treatment. However, because these treatment modalities are associated with increased local toxicity, effective treatments of local acute reactions would be valuable.

Numerous medical agents, such as beta-carotene [283], chlorhexidine [284, 285], prostaglandin E1 [286], benzyldamine [287], glutamine [288], povidone-iodine [289], hydrogen peroxide rinses [290], sucralfate [291, 292] and local antibiotics [293, 294], have been tested for their ability to alleviate radiation-induced mucositis. The results have been disappointing, and in a recent meta-analysis of randomized clinical trials on mucositis prophylaxis reported by Sutherland and Browman, the conclusion was that at present insufficient evidence exists to support the development of recommendations for the prevention of oral mucositis in clinical practice, with the possible exception of narrow-spectrum antibacterial agents [295]. In a recent double-blind,

randomized phase III study, antibiotic lozenges did not have a significant impact on the severity of radiation- induced mucositis [296].

Over the last few decades the radioprotective activity of thiol-containing compounds has been under investigation. The most promising of these compounds has been amifostine. Its mechanism of action in radioprotection is thought to take place through scavenging of radiation-induced free radicals [297]. In small clinical trials, amifostine was found to protect against radiation-induced salivary gland damage as well as against radiation-induced acute mucositis [298, 299]. In a phase III randomized trial by Brizel *et al.*, 315 patients with head and neck cancer were randomized to radiation treatment with or without amifostine at a daily infusion of 200 mg/m² 15 to 30 minutes before radiotherapy. A significant reduction in radiation-induced xerostomia was observed, but amifostine did not diminish the severity of acute mucositis [48].

Based on preliminary reports with small patient numbers, granulocyte-macrophage colony-stimulating factor (GM-CSF, molgramostin) was considered as one of the most promising new agents for the prevention of radiation-induced mucositis [300-302]. GM-CSF is a glycoprotein with a molecular weight of 22 kD. GM-CSF acts by enhancing colony formation of granulocytes, macrophages and eosinophils and it also regulates several functions of mature leucocytes, macrophages and dendritic cells in the dermis and submucosa [303, 304]. In addition, GM-CSF enhances keratinocyte and fibroblast growth and improves healing of cuts, leg ulcers and skin grafts [305-310]. In early reports, subcutaneously given GM-CSF was found to significantly alleviate radiation-induced mucositis [311, 312]. This could not, however, be confirmed in a small randomized study, where subcutaneous GM-CSF was associated with moderate toxicity [312]. A few small, non-randomized studies have suggested that GM-CSF mouthwashes during oropharyngeal radiotherapy might reduce the severity of radiation-induced mucositis [300-302].

Before being adopted in clinical practice, the influence of topical GM-CSF must be confirmed in adequately powered randomized studies.

In the absence of specific anti-mucositic agents for radiation-induced acute mucositis, the mainstays in the care of head and neck cancer patients who receive radiotherapy are careful oral hygiene during radiotherapy and adequate nutritional protocols to maintain a good nutritional status during the radiotherapy course. In recent trials, percutaneous endoscopic gastrostomy (PEG) has been demonstrated to be feasible and effective in preventing dehydration and malnutrition during a course of head and neck radiotherapy [313-315].

4.4.2. Prevention of xerostomia

The most important aspect in the prevention of radiation-induced salivary gland damage is careful treatment planning to minimize the volumes of salivary glands included in the irradiation fields. Based on the normal secretion patterns of saliva, most of the stimulated saliva flow will be maintained if the parotid gland dose can be limited, and avoidance of submandibular gland irradiation at high doses will help to maintain the unstimulated saliva production.

As salivary glands are considered to function as parallel organs with respect to late radiation-induced damage, preservation of the salivary function is to be expected if irradiation of large volumes of the major salivary glands can be avoided. In a study by Roesink *et al.*, an increase of the irradiated parotid gland volume from 0-40% to 90-100% while maintaining a mean parotid dose of 35 to 45 Gy resulted in a decrease in the saliva flow ratio from approximately 100% to less than 10% following irradiation [316]. CRT and especially IMRT provide new possibilities for

defining the volumes of salivary glands irradiated and the doses received by each gland. Computer-based radiotherapy planning enables the use of dose-volume histograms (DVHs), which allow for more accurate estimations of the target volumes and doses received by each organ [97, 254].

Eisbruch *et al.* recently reported that cumulative doses of less than 24 to 26 Gy to the main salivary glands are associated with substantial preservation of saliva flow rates [317]. In another study, no treshold dose was found, and limiting the cumulative parotid gland dose to 39 Gy or less was recommended [316]. Not only restricting the dose to the major salivary glands but also limiting the dose to the minor salivary glands is considered important, since the minor glands produce up to 70% of the total mucins secreted by salivary glands [318].

The most effective radioprotective compound studied in prevention of radiation-induced xerostomia has been amifostine. Amifostine has a cytoprotective activity only when present during exposure of cells to radiation or cytotoxic agents [297]. Amifostine is administered as an inactive prodrug and is activated extracellularly by alkaline phosphatase, which is present at high concentrations in normal but not in malignant tissues [319-321]. The effectiveness of amifostine in prevention of radiation-induced xerostomia was shown in a large randomized trial by Brizel *et al*. In this study, the incidence of xerostomia at one year post-treatment was significantly reduced in patients who received amifostine (34% vs. 57%) [48]. The side-effects associated with amifostine are nausea, vomiting and hypotension. Allergic reactions, sometimes resulting in life-threatening anaphylactoid reactions or toxic epidermal necrolysis, have been described [322, 323]. In a phase III study by Rades *et al.* [324] intravenous amifostine was given to 39 patients receiving radiotherapy for head and neck cancer; nine of the patients received also concurrent chemotherapy. Intravenous amifostine had to be discontinued in 16 (41%) out of the 39 patients and in 7 (78%) out of the 9 patients who received concomitant chemoradiotherapy, which led to discontinuation of the study.

Based on this study and other data from the literature, discontinuation of amifostine occurs in about 24% of the patients who receive amifostine concurrently with radiotherapy. Subcutaneous administration of aminofostine has been reported to be associated with less toxicity than intravenous administration. The frequency of severe adverse events remains nevertheless over 10% [325, 326].

Another widely investigated drug in prevention and treatment of radiation-induced xerostomia is pilocarpine. Pilocarpine is a muscarine cholinerginic agonist that increases salivary output in normal conditions [261]. In a randomized, placebo-controlled trial by Johnson *et al.*, pilocarpine was shown to significantly improve the sensation of oral dryness following radiation therapy, but it did not have any permanent impact on quantitative measures of salivary flow [327]. In a non-randomized study by Zimmerman *et al.*, the use of 5 mg oral pilocarpine four times a day during radiotherapy for head and neck cancer and 3 months afterwards was found to be associated with significantly less subjective xerostomia than reported in retrospective controls [328]. Similar results have obtained from one small randomized study [329].

Theoretically, by combining IMRT and amifostine, greater sparing of the salivary function might be achieved than by either treatment modality alone [330, 331]. A randomized trial is ongoing on subcutaneously delivered amifostine in patients who receive IMRT for head and neck cancer, but the results are not yet available [331].

5. AIMS OF THE STUDY

The general objective of this study was to improve treatment efficacy and to reduce treatment-related adverse effects in squamous cell head and neck cancer. The specific aims were as follows:

1. To study expression of cyclin A and the Ki-67 antigen as predictors for locoregional recurrence and outcome in laryngeal cancer patients treated with surgery and postoperative radiotherapy.

2. To evaluate the effects of tumour cell repopulation and radiotherapy treatment time on local control in T1 laryngeal cancer treated with radiotherapy.

3. To assess the efficacy of biweekly escalated, accelerated hyperfractionated radiotherapy with concomitant single-dose mitomycin C in the treatment of advanced laryngeal and hypopharyngeal cancers with respect to tumour control and organ preservation.

4. To estimate the effect of granulocyte-macrophage colony-stimulating factor in the prevention of radiation-induced mucositis during postoperative radiotherapy of squamous cell cancer head and neck.

5. To study intensity-modulated radiotherapy in the prevention of radiation-induced salivary gland damage and subsequent xerostomia

6. PATIENTS AND METHODS

6.1. PATIENTS (STUDIES I-V)

The patient population involved in these studies comprised of 285 patients with squamous cell head and neck cancer treated by definitive or postoperative radiotherapy at the Department of Radiotherapy and Oncology, Helsinki University Central Hospital in 1982 to 2002. The patients involved in Studies I to V are given in Table 3.

Table 3. Patients and the treatments given in Studies I-V.

Study	Number of Patients (F, female; M, male)	Year of Diagnosis	Clinical Stage	Type of Radiotherapy	Scheduled concomitant medication
I	90 (F 12, M 78)	1982 to 1998	Stage II-IV, T1-4 N0-2 M0	Postoperative RT (split-course, N=56; continuous, N=34)	None
II	117 (F 11, M 106)	1982 to 1993	Stage I, all T1N0 M0	Definitive RT (split-course, N=89; continuous, N=28)	None
III	21 (F 3, M 18)	1998 to 2001	Stage III-IV, T2-4 N0-3 M0	Escalated, accelerated, hyperfractionated RT	Mitomycin C 10mg iv given on d. 30
IV	40 (F 21, M 19)	1999 to 2001	Stage II-IV, T1-4 N0-N2 M0	Postoperative RT	GM-CSF 37.5µgx 4 vs. Sucralfate 1g x4 mouthwashes
V	17 (F 10, M 7)	2000 to 2002	Stage II-IV, T1-4 N0-2 M0	Intensity modulated Radiotherapy (IMRT)	None

In Study I, paraffin-embedded tumour samples from 90 laryngeal cancer patients (78 men, 12 women) were stained for cyclin A and the Ki-67 antigen using immunohistochemistry. All of the patients were treated with partial or total laryngectomy followed by postoperative radiotherapy to a total dose of 50 Gy or greater. The median age at diagnosis was 63 (range 35 to 85 years). The median post-treatment follow-up time was 91 months (minimum 48 months).

Study II was an analysis of the effect of radiotherapy treatment time on the local tumour control in patients with T1 laryngeal cancer patients. The patient series consisted of 117 consecutive patients (106 men, 11 women) with T1 laryngeal cancer treated between 1982 and 1993 by radical radiotherapy alone, given either as continuous (n=28) or split-course treatment (n=89). The median age of patients was 66 (range, 42 to 87 years). Eighty cancers were located in the mobile vocal cord, 33 involved the anterior commissure and four involved both cords.

In Study III, 21 patients (18 men, 3 women) with stage III to IV laryngeal or hypopharyngeal squamous cell cancer were treated with a biweekly escalated, accelerated hyperfractionated radiotherapy schedule with a concomitant single dose of mitomycin C. Ten of the patients had laryngeal and 11 hypopharyngeal cancer. Their median age was 59 (range, 27 to 71) years.

Study IV was a double-blind, prospective, randomized study that compared granulocyte-macrophage colony-stimulating factor (GM-CSF) mouthwashes with sucralfate mouthwashes in the prevention of radiation-induced mucositis. Forty patients (27 men, 13 women) with radically operated head and neck cancer were randomly allocated to use either GM-CSF (n=21) or sucralfate (n=19) mouthwash during postoperative radiotherapy. Patients with prior chemotherapy or radiotherapy, chronic autoimmune or inflammatory disease or the World Health Organization (WHO) performance status >2 were not eligible for the trial.

In Study V, 17 patients (10 women, 7 men) with primary squamous cell cancer of the head and neck were treated with IMRT. A decision to use IMRT instead of conventional conformal radiotherapy was made in these cases, because the use of conventional radiation therapy would have resulted in irradiation of all major salivary glands to a cumulative dose greater than 45 Gy with a high risk of subsequent post-irradiation xerostomy. The mean age of the patients was 55 (range, 32 to 78) years. The WHO performance status was 0 (normal) in 11 cases and 1 in six patients. Six patients had a nasopharyngeal primary tumour and the remaining 11 had oropharyngeal cancer. Three of the patients had stage II, four had stage III, and 10 stage IV cancer. Eleven patients received definitive radiotherapy with a curative intent, and 6 patients received postoperative radiotherapy.

In studies III and IV, which required scheduled concomitant medication, the study protocol was approved by an Ethical Committee of the Helsinki University Central Hospital before enrolling any patients in the trials, and the patients provided a written informed consent. Studies I, II and V were based on retrospective analyses of hospital case records and data collected along with standard care of patients.

6.2. TREATMENT (I-V)

The patients and treatments in Studies I-V are summarized in Table 3. All 90 patients in Study I underwent surgery followed by postoperative radiotherapy. Total laryngectomy was performed in 72 (80%), supraglottic laryngectomy in 14 (16%), and hemilaryngectomy in 4 (4%) cases. Neck dissection was done in 25 cases (28%) where nodal metastasis was suspected. In 23 of these,

presence of nodal metastases was confirmed histologically. After surgery all patients received postoperative radiotherapy. The median time from surgery to the beginning of radiation therapy was 47 (range, 23 to 83) days. The first 26 patients were treated with ^{60}Co between 1981 and 1987, and the following 64 patients with a 6 MV linear accelerator between 1988 and 1993. The upper neck was irradiated through two lateral portals, and the lower neck from a separate anterior field. The total dose varied from 50 to 66 Gy depending on the size of the primary tumour and involvement of surgical margins. The fraction size ranged from 1.8 to 2.0 Gy, and in all patients, the treatment was given in one daily fraction. Between 1981 and 1993, the treatment for 56 cases was given as split-course radiotherapy with a 14- to 21-day planned gap in the middle of the radiotherapy, between 1994 an 1998 as continuous radiotherapy for the remaining 34 cases. The mean total treatment time in patients treated with split-course radiotherapy was 66 days (range, 58 to 71), and in those treated with continuous radiotherapy 41 days (range, 33 to 41).

All 117 patients included in Study II received definitive radiotherapy for TI laryngeal cancer. Sixty-six of these patients were treated with ^{60}Co, and 51 with a 6 MV linear accelerator. Two lateral opposing fields with compensatory wedges were used in all patients. The target dose was calculated by a computer-based radiotherapy planning program. The first 89 patients treated between 1982 and 1987 received split-course radiotherapy with a planned 2- to 3 week gap in the middle of the radiotherapy course. The mean total dose in this group was 66 Gy (range, 60 to 68.2) and the overall treatment time varied from 53 to 79 days (mean, 65). The fraction size was increased by 10% from 2.0 to 2.2 Gy to compensate for the planned gap. Since 1988, 28 patients were treated with a continuous radiotherapy course. In these patients, the mean total dose was 62 Gy (range, 60 to 66), and the mean overall treatment time was 44 days (range, 39 to 50). All patients received the therapy in 2-Gy daily fractions.

In Study III a hyperfractionated, escalated radiotherapy schedule with concomitant MMC was used in all 21 cases. An individually made thermoplastic mask (Sinmed®) was used for head and neck fixation. Treatment planning was carried out using a CT-based treatment planning computer program (Cadplan®, version R.6.2.7., Varian Medical Systems). Radiotherapy doses were prescribed according to the ICRU 50 specifications [332], where the ICRU reference point was chosen centrally within the planning target volume (PTV). At the beginning of the radiotherapy course, the treatment volume encompassed the primary tumour and the locoregional lymphnodes. Radiotherapy was given in 2 daily fractions with an interfraction interval of at least 6 hours. The fractionation schedule is presented in Table 1 of the original contribution of Study III. After a cumulative dose of 52 Gy, the fields were reduced and the macroscopic tumour plus 1- to 2 cm margins were boosted to a total cumulative dose of 74.4 Gy. A single dose of MMC 10mg/m^2 was given intravenously 2 hours before irradiation on day 30 of the radiation course. A part from a diagnostic biopsy, none of the patients underwent primary surgery. According to the treatment protocol, surgery on the primary site was required only when the tumour persisted for longer than 2 months after the chemoradiotherapy or when the tumour recurred locally. A radical neck dissection was to be carried out after chemoradiotherapy when the clinical nodal classification was N3 or if the enlarged lymph nodes persisted 2 months after the completion of chemoradiotherapy.

Study IV was a double-blind, prospective randomized, phase III study comparing the effectiveness of granulocyte-macrophage colony-stimulating factor (GM-CSF) and sucralfate mouthwashes in the treatment of radiation-induced mucositis. All patients had undergone radical surgery for head and neck cancer and were scheduled to receive postoperative radiotherapy. Radiotherapy dose planning was performed with a CT-based planning program (Cadplan®). Treatment to the primary resection area and upper neck lymphatics was usually given through two parallel opposed fields; the lower neck lymphatics were, whenever necessary, treated from a separate anterior field. The dose to the

medulla was restricted to 38 Gy, following which, the radiation therapy to the dorsal neck was completed with 9 MeV electrons. The total dose to the primary tumour site was 50 to 60 Gy, and 50 Gy was delivered to the locoregional lymphatics. Radiotherapy was given to all patients as continuous therapy in 2-Gy daily fractions 5 times a week.

The GM-CSF mouthwash solution was prepared by dissolving 150 µg of dry drug powder into 100 ml of sterile water, and the sucralfate solution by dissolving 4.0 g of sucralfate in the same amount of water. The respective mouthwashes were started once a cumulative total dose of 10 Gy was reached (after the first week of radiotherapy) and continued until the end of the radiotherapy. Mouthwashes were used during treatment days; their use was interrupted on Saturdays and Sundays. In both treatment groups, the patients were instructed to use 100 ml of the treatment solution divided into 4 equal 25 ml doses during each day of radiotherapy. The dose of GM-CSF per one mouthwash thus was 37.5 µg, and that of sucralfate 1 g.

In Study V intensity-modulated radiotherapy (IMRT) was used to avoid the permanent xerostomia often accompanying radiotherapy of head and neck cancer. The patients were immobilized during the radiotherapy using either a conventional thermoplastic device or, in the last 7 patients of the study using a stereotactic head and neck immobilization device. The IMRT technique used to irradiate the primary tumour and the cervical lymph nodes consisted of 5 to 7 coplanar fields. In most cases (n=14) 6 fields were used. The arrangement of the fields is described in Table 2 of the original contribution. The treatment plans were generated using an inverse planning software. The dose constraints were adjusted according to the clinical situation. The cumulative dose to the spinal cord was kept under 40 Gy. Those salivary glands that were excluded from the primary target volume were included in the optimization procedure using a maximum dose constraint of 25 Gy in the first 5 patients, and 16 to 20 Gy in the remaining 11 patients. In addition, a volume of healthy

tissue was delineated outside the PTV in each CT slice, and in CT slices located immediately cranial and caudal to the slices containing the PTV, to prevent hot spots outside the PTV. The contralateral parotid gland was treated in the optimization procedure as an organ-at-risk (OAR) in all patients, and in 6 patients the contralateral submandibular gland was also excluded from the target volume.

6.3. IMMUNOHISTOCHEMISTRY (STUDY I)

Immunohistological analysis was performed on tissue sections prepared from formalin-fixed, paraffin-embedded archival tissue of the excised primary tumour; no needle biopsy samples were included. The tissue sections were deparaffined in xylene, and the samples were rehydrated using an ethanol series. Antigen demasking was carried out by heating the samples in a microwave oven in 0.1 M citric acid buffer at pH 6, 4 times for 5 minutes. For immunohistochemistry, the specimens were incubated overnight at room temperature with an anti-human cyclin A mouse monoclonal antibody at a dilution of 1:100 (Novocastra Laboratories Ltd., Newcastle, UK). The sections were counterstained with haematoxylin and eosin, and mounted. Hyperplastic human tonsillar tissue was used as a positive control; the primary antibody was omitted in the negative control samples. Immunostaining for the Ki-67 antigen was performed similarly to staining for cyclin A. A rabbit anti-human antibody (A 0047; DAKO A/S, Glostrup, Denmark) at a dilution of 1:100 was used as the primary antibody.

The assessment of the staining was done similarly as described elsewhere[179]. In brief, in each case, a total of 5 fields were assessed at a magnification of 10 x 40 using a Leitz Laborlux D microscope (Wetzlar, Germany). The fields were chosen from the tumour areas showing the highest

density of positive nuclear staining when scanned at a low magnification. To determine the percentage of positively staining nuclei, an ocular grid consisting of 100 (10x10) squares was used. All positive nuclei from the grid area were counted. To estimate the number of negative nuclei within the same 100-square field, we counted the number of nuclei in 3 non-adjacent rows of 10 squares, and multiplied the mean score by 10. For both cyclin A and Ki-67 staining the results are reported as the percentage of tumour cells with positive nuclear staining.

6.4. RANDOMIZATION (STUDY IV)

Study IV was a double-blind, prospective, randomized phase III study in which 40 patients were scheduled to receive postoperative radiotherapy for head and neck cancer. They were randomized to receive either GM-CSF or sucrafate mouthwashes during the radiotherapy. Randomization was done using computer-generated random digits; 21 patients were assigned to GM-CSF mouthwashes and 19 to sucralfate mouthwashes to be given during radiotherapy.

After the patients had provided a written informed consent, they were assigned to a treatment group. The patient’s name and social security number were provided for unequivocal indentification of each patient. The patients were stratified before randomization by the volume of oral cavity and oropharyngeal mucosa involved in the radiotherapy target volume to guarantee that the mucosal areas irradiated were roughly equal in both study arms. The stratification was done on whether 50 to 75% or over 75% of the oropharyngeal mucosa was included in the target volume.

6.5. ASSESSMENT OF MUCOSITIS (III-V)

The Radiation Therapy Oncology Group acute radiation morbidity scoring criteria were used in the estimation of the degree of radiation mucositis in Studies III-V [255]. In Study III, the examination of the degree of mucositis and assessment of mucositis-related symptoms was done before the beginning of radiotherapy, weekly during therapy, and 1, 2 and 4 weeks after therapy. Oral mucositis-related pain was estimated by the patients themselves using a linear visual analogue scale (VAS) [333] from 0 to 10, where 0 represents absence of pain and 10 maximal pain. Grave mucositis is associated with worsening of the patients' nutritional status, and therefore patients' weight and serum prealbumin levels were monitored in addition to the clinical status to obtain an objective measure of the nutritional status during the radiotherapy course. The biological half-time of prealbumin is short (about 2 days), and when the protein balance is negative, the serum prealbumin level falls rapidly [334, 335]. The use of local anaesthetics (lidocaine mouthwashes) and systemic analgesic drugs was registered for each patient as was the use of antibiotics or antimycotics for mucositis-related infections. Mucositis-related interruptions in radiotherapy and possible hospitalization of trial patients during radiotherapy were also recorded.

6.6. ASSESSMENT OF LARYNGEAL FUNCTION (STUDY III)

Laryngeal function following radiotherapy was assessed by performing videolaryngoscopy and the voice quality was evaluated perceptually at the Department of Otorhinolaryngology – Head and Neck Surgery of the Helsinki University Central Hospital.

6.7. ASSESSMENT OF XEROSTOMIA (V)

In Study V, the salivary gland function of patients treated with IMRT was assessed before the beginning of radiotherapy and at 6 and 12 months after radiotherapy had ended. Both the basal and the stimulated saliva secretion rates were measured. The basal rate was obtained by measuring the total saliva secretion over 15 minutes, after which saliva secretion was stimulated by chewing a piece of paraffin wax, and collected for 15 minutes. The patients prepared the paraffin wax by chewing for 2 minutes before saliva collection. The patients were advised not to eat, drink or smoke for one hour before collection of stimulated saliva was initiated. In addition to measuring saliva secretion, the degree of xerostomia was also assessed by grading according to the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) classification systems [255].

6.8. STATISTICAL ANALYSES

Data in Studies I and III to V were analysed using the Number Cruncher Statistical System (NCSS) 2000 program. In Study II, the BMDP (Biomedical Computer Programs-P series) statistical package was used.

The Kaplan-Meier product-limit method was used to estimate the survival distributions for disease-free survival and overall survival (I to III). Comparisons of the survival rate between groups were done using the log-rank test or univariate Cox regression analysis (I). The relative importance of prognostic factors was analysed using Cox's proportional regression model (I). The association between Ki-67 and cyclin A expression was investigated using the Spearman correlation test, and associations of cyclin A and Ki-67 expression with categorical parameters were assessed with the Mann-Whitney test or the Kruskal-Wallis analysis of variance (I). The Mann-Whitney test was also

used in Studies IV and V to test differences between treatment groups. Frequency tables were analysed using either chi-square or Fisher's exact test (I, IV). In study IV, repeated observations were analysed with repeated measures analysis of variance. All p-values are 2-sided.

The analysis of the effect of the overall treatment time on local control and the estimation of proliferation rates are presented in Section 2.5. of the original contribution (II). The mathematics for modelling of salivary flow as a function of dose is presented in detail in the Appendix of Study V.

7. RESULTS

7.1. EFFECT OF THE OVERALL TREATMENT TIME AND CELL REPOPULATION ON THE FREQUENCY OF LOCOREGIONAL RECURRENCE (I-III)

In Study I, the effect of the overall radiotherapy treatment time on tumours with varying cell proliferation rate was examined. Cancers with high Ki-67 expression (>34% nuclei positive, the highest tertile) recurred more frequently locoregionally when treated with split-course radiotherapy than when treated with a continuous course of therapy (p=0.035), whereas with lower Ki-67 expression, a break in treatment had no influence on the frequency of locoregional recurrences (p=0.93). Seventeen (30%) of the 56 patients treated with split-course radiotherapy and 6 (18%) of the 34 patients treated with a continuous radiation therapy course had a local recurrence (p=0.18). When the proliferative fraction of the cancers was taken into account, planned gaps in the radiotherapy course turned out to be more deleterious for patients who had cancer with a large proliferative fraction. Only one locoregional recurrence was observed among the 12 (8%) patients who had high Ki-67 expression cancer when treated with continuous radiotherapy as compared with 8 (44%) among the 18 patients treated with planned split-course radiotherapy. In contrast, the proportion of locoregional recurrences was roughly similar in patients with cancer with low Ki-67 expression irrespective of whether a planned break was held or not (24% vs. 23%, respectively, p=0.93).

Patients with a high cyclin A expression level had somewhat more locoregional recurrences when treated with split-course radiotherapy, but this difference did not reach statistical significance. When both tumour cyclin A and Ki-67 expression were within the highest tertile, only one of such patients (17%) treated with continuous radiotherapy had a locoregional recurrence, whereas as

many as 8 (73%) of 11 of such patients treated with split-course radiotherapy had a local recurrence (p=0.050). No significant associations were found between the duration of the surgery-to-radiotherapy interval, tumor proliferation fraction, and the frequence of locoregional recurrences.

The impact of tumour cell repopulation on outcome of T1 laryngeal cancers treated with radiotherapy was evaluated in Study II. The local control rate was 95% (range, 94 to 96%) for the continuous and 81% (range, 75 to 91%) for the split-course therapy groups, respectively. During follow-up a total of 18 recurrences took place, all within the first 3 years following radiotherapy. The median overall duration of radiotherapy was 65 days for patients who had recurrence as compared with 59 days among those with no recurrence. The D_{prolif} value at the steepest part of the response versus time curve was 0.48 Gy/day for local control. The dose required to compensate for a one-week increase in treatment time for local control at the 90% level at 3 years was 3.5 Gy.

In Study III, the treatment schedule was planned to compensate accelerated tumour cell repopulation during radiotherapy. At the end of chemoradiotherapy 10 (48%) patients had complete primary tumour clearance and 11 (52%) patients had a partial response. When the responses were evaluated 2 months after therapy by clinical examination and by laryngomicroscopy, all patients showed a complete response with no residual primary tumour. With a median follow-up of 48 (range, 28 to 61) months, a local control rate of 70% and a disease-free survival rate of 60% were achieved in the laryngeal cancer patients; whereas in patients with hypopharyngeal cancer, the corresponding figures were 64% (82% after salvage surgery) and 36%.

7.2. CORRELATION OF CYCLIN A AND Ki-67 EXPRESSION WITH OTHER CLINICOPATHOLOGICAL FACTORS AND SURVIVAL (I)

Cyclin A and Ki-67 expression as predictors of locoregional recurrence and survival in laryngeal cancer treated with surgery and postoperative radiotherapy was evaluated in Study I. The median of 14% (range, 0 to 81%) of tumour cells expressed cyclin A and 25% (range, 0 to77%) expressed the Ki-67 antigen. A strong positive correlation was found between immunostaining for cyclin A and Ki-67 (r_s = 0.79, p=0.002). Supraglottic cancers had a higher frequency of cells staining positively for cyclin A and Ki-67 than glottic cancers, suggesting that, in general, the proliferative compartment is larger in supraglottic cancer (p=0.008 and p=0.006, respectively). A high percentage of nuclear Ki-67 staining was associated with a poor histological grade of differentiation (p= 0.0009), and a similar trend was found for cyclin A (p=0.09). Neither cyclin A nor Ki-67 showed a significant association with the primary tumour size, nodal status, clinical stage or presence of positive margins at surgery.

High cyclin A expression (>19% positive cancer cell nuclei, the highest tertile) was found to be associated with a high rate of locoregional tumour recurrence and unfavourable disease-free and overall survival as compared with cases with a lower expression (p=0.025, p=0.032, and p=0.042, respectively). In a multivariate analysis, high cyclin A expression was an independent predictor of poor disease-free survival (RR 2.4, 95% CI 1.2-4.9, p = 0.013) and overall survival (RR 2.1, 95% CI 1.2-3.6, p=0.012) together with a Karnofsky's performance status and the presence of positive margins at surgery. Thus, these findings suggest that cyclin A may be a novel prognostic factor in laryngeal cancer.

7.3. SAFETY AND FEASIBILITY OF MITOMYCIN C GIVEN CONCOMITANTLY WITH ACCELERATED, HYPERFRACTIONATED RADIOTHERAPY (III)

Although toxicity of the combination therapy was substantial, only one grade 4 toxic adverse event was encountered (skin necrosis, size 2x2 cm). In 6 patients, the skin reactions were recorded as grade 3, and in the rest of the patients as grade 1 or 2. The skin reactions were observed mainly at the site of the primary tumour and in the upper neck, where the radiation dose was highest. No grade 4 mucosal reactions were noted (grade 3, 62%; grade 2, 38%). Twelve (57%) patients were hospitalized for nutritional support, and 5 of these needed a nasogastric tube, which could be removed in all cases within a few weeks following the radiotherapy. Acute reactions healed within 3 months from the last day of radiotherapy. No MMC- related haematological toxicity was registered. In general, the overall toxicity was considered to be acceptable, and the treatment regimen feasible to administer.

7.4. GRANULOCYTE-MACROPHAGE COLONY-STIMULATING MOUTHWASHES IN PREVENTION OF RADIATION-INDUCED MUCOSITIS (IV)

Oral mucositis tended to be less severe in the group of patients who received GM-CSF than those given sucralfate mouthwashes (p=0.072). Complete (n=1) or partial (n=4) healing of mucositis occurred during the radiotherapy course in 5 (24%) patients in the GM-CSF group, but in none in the sucralfate group (p=0.049). Patients who received GM-CSF had less mucosal pain (p=0.058) and were less often prescribed opioids for pain (p=0.042). Three patients in the sucralfate group needed hospitalization for mucositis during radiotherapy compared with none in the GM-CSF group. Four (21%) patients in the sucralfate group and none in the GM-CSF group required an interruption in the radiotherapy course (p=0.042). No significant differences in weight, the prealbumin level or the blood cell counts were found between the groups, and both mouthwashes

were well tolerated. These findings suggest that GM-CSF mouthwashes may decrease the severity of radiotherapy-induced mucositis when administered during radiotherapy.

7.5. LARYNGEAL FUNCTION (III)

All patients with hypopharyngeal cancer had a well functioning larynx including a good voice quality after radiation therapy. Of the 6 laryngeal cancer patients surviving with a preserved larynx, the voice quality of was deemed to be good in 3, slightly hoarse in one and poor in 2. Although based on small patient numbers, these findings suggest that many hypopharyngeal cancer patients have useful laryngeal function following larynx-preserving therapy.

7.6. EFFECT OF SALIVARY GLAND SPARING BY INTENSITY-MODULATED RADIOTHERAPY ON RADIATION RELATED XEROSTOMIA (V)

The median basal saliva flow rate was 0.13 ml/min prior to the radiotherapy course, 0.04 ml/min at 6 months and 0.07 ml/min at 12 months after completion of IMRT in 17 patients treated with IMRT for head and neck cancer. The decline in the basal saliva flow within the first 12 months after receiving IMRT as compared with the baseline was 42% ($p=0.065$, paired t-test). The corresponding values for stimulated saliva secretion were 0.49 ml/min, 0.33 ml/min and 0.45 ml/min. This decline observed within the first 12 months following irradiation in the stimulated saliva flow rate was not significant ($p=0.32$). High cumulative mean parotid gland doses were associated with low stimulated saliva flow rates measured following radiotherapy. A D_{50} value of 26 Gy was calculated from the dose-response curve for the stimulated parotid gland saliva flow rate.

No unexpected adverse effects occurred during or after the IMRT course. All patients had mucositis during the radiotherapy (grade 1, n=1; grade 2, n=9; grade 3, n=7). Two patients were hospitalized because of mucositis. The skin reactions were mild.

During a median follow-up time of 24 (range, 12 to 40) months, none of the patients had a local cancer recurrence. Two patients developed distant metastases at 10 and 21 months following the treatment; local control was, however, maintained also in these patients.

These results suggest that the salivary gland function may be partially preserved by IMRT in the majority of patients who are at risk for developing severe xerostomia after conventional therapy without compromizing the local control rate. Hovever, the non-randomized nature of the study and the relatively short follow-up prevent making firm conclusions.

8. DISCUSSION

Optimal results in the treatment of head and neck squamous cell cancer cannot be achieved without careful selection of the treatment modalities for each patient. This is not possible without estimation of the factors that determine the prognosis. Although the TNM staging of tumours remains an important method in outcome estimation and treatment selection, evaluation of new tumour- and patient-related factors that can help to classify patients into precisely defined prognostic subgroups is a priority.

High cyclin A expression was associated with an elevated risk of local recurrence and poor disease-free and overall survival in univariate analyses in patients treated with laryngectomy and postoperative radiotherapy for laryngeal cancer. In addition, high cyclin A expression proved to be an independent predictor of DFS and overall survival in a multivariate analysis. These observations are in line with results obtained in earlier studies on a few other histological types of human cancer [174-179, 336]. Cyclin A was found to be a stronger predictor of prognosis than Ki-67 in the present study.

Cyclins, cyclin-dependent kinases and the genes regulating their synthesis may also become targets for cancer therapy in head and neck cancer. For example, a CDK-inhibitor, flavopiridol, is currently being tested in clinical trials [169, 170]. Another CDK inhibitor, CCI-779, decreases the kinase activity of the CDK4-cyclin D complex in a p53-independent fashion [337]. In line with other experiments, suppression of endogenous cyclin D1 expression in a human head and neck squamous cell carcinoma line was found to suppress *in vitro* cell growth and tumourigenicity in athymic nude mice, and antisense cyclin D1 transfection to enhance tumour cell chemosensitivity to cisplatin

[338]. Transfection of antisense cyclin D1 to CLL23 cells enhanced responsiveness to cisplatin in one study[339].

The associations between Ki-67 expression with tumour clinical characteristics and patient outcome appear to be weak and conflicting in head and neck cancer [145-147, 149]. In our study, Ki-67 did not have an association with prognosis in laryngeal cancer treated with surgery and postoperative radiotherapy. A strong correlation was, however, found between the histological grade and Ki-67 expression, and Ki-67 expression level was also significantly higher in supraglottic than in glottic cancers. In addition, our results suggest that planned gaps in the radiotherapy course may be more deleterious in the treatment of cancers with a large tumour proliferative fraction as estimated by high expression of Ki-67, or both high Ki-67 and cyclin A. Breaks in a course of radiotherapy have an adverse effect on local control in laryngeal cancer [124, 126, 128, 340]. In addition to radiotherapy treatment time, the time from surgery to the beginning of radiotherapy also has been identified to influence the outcome of head and neck cancer patients[144]. The lack of such correlation in the present study may have been due to the relatively small number of patients studied. In head and neck cancer, prolonged overall treatment times appear to worsen the outcome also in postoperative radiotherapy [121, 341]. Discovery of tumour-related factors that can predict the outcome associated with various fractionation schedules may be helpful in determining when to use the accelerated treatment protocols.

The negative impact of radiotherapy gaps on tumour control has been considered to be result from repopulation of clonogenic tumour cells. The repopulation rate picks up speed over the course of fractionated radiotherapy [46, 122]. This accelerated repopulation probably begins about 2 weeks after the start of radiotherapy [123]. For T2 to T4 laryngeal cancer, it has been estimated that due to tumour cell repopulation an additional dose of 0.5 to 0.8 Gy is needed to compensate for each day

of treatment interruption [126-130]. In our study on 117 patients with T1 glottic cancers treated with radiotherapy alone, the results showed a mean D_{prolif} value at the steepest part of the response versus time curve of 0.48 Gy for local control at 3 years. The dose required to compensate for a one-week increase in treatment time for local control at the 90% level was 3.5 Gy.

The recommendations for the optimal radiotherapy treatment duration in head and neck cancer vary; according to Wang and Efird, the overall treatment time should be shorter than 6 weeks [342], and Fowler suggested a duration of 4 to 5.5 weeks [343]. A few trials of accelerated radiotherapy suggest that shortening of the overall treatment time may provide therapeutic gains in the treatment of head and neck cancer [40, 136]. The local control rates can be improved by hyperfractionated or accelerated radiotherapy protocols as compared with standard fractionation, but this has resulted to suprisingly little, if any, improvement in the overall survival of these patients. This might be due to the relatively small improvements in the local control figures rates achieved and to increased toxicity of the treatment. Patients with SCCHN also often have concomitant diseases that strongly influence the overall survival. Adding concomitant chemotherapy to a fractionated radiotherapy schedule is likely to enhance the therapeutic gain even further [41-43, 45, 202, 344]. This advantage is probably achieved only, when the radiotherapy is given as a continuous treatment; planned gaps may negate the therapeutic effect gained by chemoradiotherapy [30, 212].

One of the chemotherapeutic agents tested in chemoradiotherapy of head and neck squamous cell cancer is mitomycin C [210-213]. MMC has been shown to be preferentially cytotoxic for hypoxic cells as compared with well-oxygenated cells [198, 208, 209]. This may be of value when treating advanced head and neck cancers, which often contain poorly oxygenated, radioresistant clonogenic cells. In our trial, single-dose MMC was added to an escalated, accelerated radiotherapy schedule to treat advanced laryngeal and hypopharyngeal cancers. Theoretically, this approach might be

effective in counteracting repopulation during a radiotherapy course and might provide a better chance of eradicating the radioresistant, hypoxic cells within these tumours. A high percentage of local control was achieved, although many patients in the hypopharyngeal cancer group ultimately died of local recurrence or distant metastases; in laryngeal cancer, the survival figures were better. This treatment schedule may also provide an opportunity to preserve the laryngeal function in most patients. No clinical trial has directly compared the effectiveness of MMC with other chemotherapeutic agents in chemoradiotherapy of head and neck cancer. The most studied agent at present is cisplatin, and a randomized trial comparing the relative effectiveness of cisplatin- and MMC-based chemoradiotherapy would be valuable. There are, however, also newer agents that have the capability of sensitizing hypoxic cells to radiation, and these may replace MMC in the future. One of these agents is tirapazamine, which is a benzotiazine bioreductive compound that has shown differential toxicity for hypoxic cells [199]. In preclinical studies, an additive effect was demonstrated when tirapazamine was combined with radiation [345]. Tirapazamine was also shown to markedly potentiate the cytotoxicity of cisplatin [346]. An early clinical report indicates that tirapazamine might be effective as a part of multiagent chemoradiotherapy regimen for head and neck cancer [347].

Accelerated radiotherapy protocols and chemoradiotherapy are capable of producing significant improvement in local control, and the latter also in survival of head and neck cancer patients. Their main disadvantage is an increase in radiation-induced acute and late normal tissue reactions. If these reactions can not be treated properly, the therapeutic gain produced by these more intense treatment protocols is reduced.

Oral and pharyngeal mucositis is the most common and clinically significant acute adverse effect of radiotherapy for head and neck cancer. When using conventional fractionation, radiation-induced

mucositis usually appears during the second week of radiation and then proceeds from enanthema to spotted or confluent pseudomembranous mucositis [142, 260, 270, 271]. Acute mucosal reactions cause pain, and difficulties in swallowing and speaking. Difficulties in eating may lead to a poor nutritional status and weight loss. Mucositis also predisposes to local and systemic infections. Severe mucosal reactions are the predominant cause for interruption of radiotherapy for head and neck cancer, which can result in significant loss of the tumour control probability [46, 47]. Thus far, none of the numerous agents tested for prophylaxis of radiation-related mucositis has demonstrated satisfactory efficacy [295]. Parenterally administered GM-CSF has been effective for oral mucositis occurring in association with cancer chemotherapy and myeloablation [348, 349]. In a prospective, randomized trial, GM-CSF mouthwashes resulted in a significantly shorter duration and quicker resolution of oral mucositis after cancer chemotherapy than the combined topical use of an antiseptic agent and amphotericin B [350]. When used for radiation-induced mucositis, subcutaneous GM-CSF failed to prove effective [312]. A few non-randomized trials have suggested that GM-CSF mouthwashes might be effective in the prevention of radiation-induced mucositis [300, 311].

In our study on GM-CSF mouthwashes versus sucralfate washes, radiation mucositis-related symptoms, body weight, serum prealbumin levels, and the blood cell counts were monitored weekly. Oral mucositis tended to be less severe in the GM-CSF group. Complete or partial healing of mucositis occurred during the radiotherapy course in 5 patients in the GM-CSF group and in none in the sucralfate group. Patients who received GM-CSF also had less mucosal pain and were less often prescribed opioids for pain. Three patients in the sucralfate group needed hospitalization for mucositis during radiotherapy compared as with none in the GM-CSF group. Four patients in the sucralfate group and none in the GM-CSF group required an interruption in the radiotherapy course. No significant differences in weight, prealbumin level or the blood cell counts were found

between the groups, and both mouthwashes were well tolerated. A similar degree of weight loss and comparable changes in the serum prealbumin levels between the two groups may suggest a lack of efficacy for GM-CSF mouthwashes in correcting the nutritional status despite some effect on mucositis. The best method to administer GM-CSF mouthwashes has not yet been described. The 3-minute mouth rinsing time used in this trial may have been suboptimal, resulting in too brief exposure of the mucosal membranes to GM-CSF. In addition, the medication was started after the first week of RT; it might have been more efficient to start it at the same time with radiotherapy. We do not know whether using the medication also on weekends might have improved the results. The GM-CSF rinses were well tolerated with no observed toxicity. The main disadvantage of the medication may be economical; at the dosages applied, the use of GM-CSF adds approximately 1000 euros to the costs of five weeks of fractionated radiation therapy in Finland in 2004 (3250€ vs. 2250€).

In high-dose radiotherapy of advanced tumours, the late reactions of the surrounding normal tissues are dose-restricting, and the late sequelae of radiotherapy can cause the patient considerable distress. The therapeutic ratio of radiotherapy may be improved by new radiotherapy techniques such as IMRT. Clinical benefits of IMRT are expected to be most pronounced at the body sites where sensitive normal tissues surround or are located close to a target with a complex 3D shape. In the head and neck region, the tolerance of many organs, including the spinal cord, the optic nerve, the eyes and the salivary glands, is much lower than the dose needed to eradicate squamous cell cancer. IMRT provides a new tool to reduce the dose to the surrounding sensitive normal structures or, alternatively, to allow dose escalation at a given level of normal tissue damage.

One of the most common and distressing adverse effects of head and neck radiotherapy is permanent xerostomia resulting from radiation-induced salivary gland damage. Parotid gland

salivary flow is markedly reduced following a cumulative dose of 30 to 50 Gy using conventional fractionation [265, 277, 278]. In Study V we observed that marked sparing of the parotid gland function can be obtained with IMRT without compromising locoregional control in the treatment of locally advanced oropharyngeal and nasopharyngeal carcinomas. The dose-response curve for stimulated parotid gland function gave a D_{50} value of 25.5 Gy. No unexpected side-effects occurred during the IMRT and a median follow-up time of 24 months. Of note, no local recurrences were observed. Thus, IMRT is a promising method for maintaining the salivary gland function without increasing the risk of local tumour recurrence.

In summary, succesful treatment of head and neck squamous cell cancer often requires a carefully planned combination of different treatment modalities to achieve optimal tumour control at a minimal level of side-effects. At present, advanced tumours appear to be best treated with continuous-course radiotherapy combined with concurrent chemotherapy. The optimal chemoradiotherapy schedules and the most effective chemotherapy agents to be used remain to be determined. In radiotherapy for head and neck cancers, giving the radiotherapy as a continuous treatment whenever feasible, with no gaps, is essential. Novel markers of cell proliferation may be helpful in defining the optimally fractionated treatment schedule for individual patients. Advanced radiation technologies, including IMRT, can provide high-dose, tissue-sparing radiotherapy to the head and neck region. New mucosa protectants, including GM-CSF, might also prove useful in the treatment of head and neck cancer.

9. CONCLUSIONS

1. Cyclin A is a novel prognostic factor for locoregional control and survival in laryngeal cancer patients treated with surgery and postoperative radiotherapy. Planned gaps in the radiotherapy course are likely to be more deleterious in patients who have cancer with a high tumour cell proliferation fraction. Immunostaining for the Ki-67 antigen may also be useful in identifying such patients.
2. Planned splits should be avoided also in the treatment of small laryngeal cancers. Each day of treatment interruption necessitates a compensatory dose of about 0.48 Gy to be added to the total cumulative radiation dose.
3. A chemoradiotherapy protocol with biweekly escalated, accelerated radiotherapy combined with single-dose MMC is feasible when given with adequate supportive care, and is effective in treatment of advanced laryngeal or hypopharyngeal cancer. Randomized trials are, however, needed to confirm these results and to compare this protocol with other existing chemoradiotherapy schedules.
4. GM-CSF mouthwashes may be moderately effective in the prevention of radiation-induced mucositis. Their use may lead to less frequent radiotherapy course interruptions due to mucositis. These findings, based on small patient numbers, require confirmation before GM-CSF mouthwashes can be recommended for routine clinical use.
5. Much of the salivary gland function can be preserved by using IMRT without loss of local control in radiation therapy of locally advanced oropharyngeal and nasopharyngeal cancer.

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